NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES (U.S.)
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ANNUAL REPORTS

DIVISION OF INTRAMURAL RESEARCH

OCTOBER 1, 1985 TO SEPTEMBER 30, 1986

PREFACE

The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) conducts and supports research on many of the most serious diseases affecting the public health. The Institute's mission includes basic and clinical research on a wide array of diseases, among which are diabetes, endocrine and metabolic disorders including cystic fibrosis; digestive diseases and nutritional disorders; diseases of the kidney and urinary tract, and blood disorders.

A focus on basic research has traditionally guided the Institute's programs. It is grounded in the belief that a fundamental understanding of biological systems will ultimately elucidate the abnormalities underlying each disease and thus is imperative for the development of the most effective strategies for prevention and therapy--and the work of the Institute involves many chronic and progressive diseases, the etiologies of which are likely to be found in the most fundamental biologic systems of the human body. In addition to basic research, the Institute also has a commitment to expand advances in the understanding of disease processes into appropriate clinical studies and ultimately into efforts to transmit knowledge and effective technologies to practicing physicians, narrowing the gap between the "bench" and the "bed" and contributing to the improvement of the nation's health.

The Institute's Division of Intramural Research has a proud tradition. Its origin can be traced to some of the oldest laboratories of the Public Health Service, and it has had a distinguished and enviable record of scientific excellence and achievement. The high caliber of our intramural research effort is reflected in the many prestigious awards, including Nobel prizes, which have come its way. Concomitantly, scientists who trained in our intramural research laboratories and branches are among the leaders of the academic community throughout the country--fostering productive collaboration with groups beyond the Bethesda campus.

This compendium chronicles ongoing intramural studies and emerging research advances, their significance, and the opportunities they present for further research. It describes a research effort of which the Institute is justly proud. We hope that this annual report for FY 1986 will engender in the reader an enthusiasm for the creative potential of our intramural scientists and for biomedical research in general.

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NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

ACTIVE PROJECTS

Z01 DK 13001-13 MRB
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ACTIVE PROJECTS (cont.)

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INACTIVE PROJECTS

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Z01 AM 19239-04 LC
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INACTIVE PROJECTS (cont.)

Z01 AM 43215-02 MD
Z01 AM 43217-02 MD
Z01 AM 55009-08 MCNE

TRANSFERRED PROJECTS

Z01 AM 17006-12 LBM
Z01 AM 18003-13 LBM
Z01 AM 18004-12 LBM
Z01 AM 18005-12 LBM
Z01 AM 19801-41 LC
Z01 AM 19803-13 LC
Z01 AM 19804-13 LC
Z01 AM 19806-11 LC
Z01 AM 21005-19 LCBG
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Z01 AM 23630-20 LBP
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TERMINATED PROJECTS

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Z01 AM 21018-04 LCBG
Z01 AM 24640-14 LBP
Z01 AM 25042-05 LCB
Z01 AM 43208-02 MD
Z01 AM 43209-02 MD
Z01 AM 43213-02 MD
Z01 AM 45036-02 CEB

Annual Report of the

Mathematical Research Branch

National Institute of Diabetes and Digestive and Kidney Diseases

Current research projects of the Mathematical Research Branch reflect a broad range of interests in the development and application of theoretical models as well as quantitative methodologies to biological systems.

This research involves several different collaborations within the Branch and with other research groups, both at the NIH and elsewhere. This report describes recent work in the areas of electrical oscillations in nerve and secretory cells, synaptic neurobiology, molecular biology, microcirculation and facilitated transport, auditory physiology, analysis of ultrasound and other imaging data, and renal physiology.

Electrical Oscillations in Nerve and Secretory Cells

Bursting:
Over the past several years we have investigated a number of theoretical models for bursting oscillations which arise in the context of cellular electrical activity as well as in physical and chemical systems. Several different mathematical mechanisms for bursting have been identified and compared to gain insight into qualitative differences in the observable burst patterns and their dependence upon physical and biological parameters. Our approach exploits the differences in time scales between fast and slow processes. First, we identify the fast and slow variables and characterize thoroughly the dynamic responses of the fast processes with the slow variables treated as parameters. We find ranges of values for slow variables such that the fast variables tend to (time-independent) steady states or to maintained oscillations; these different modes of behavior correspond to the silent and active phases of bursting, respectively. Then, we show how the slow rate processes determine a trajectory (time course) for the slow variables such that the fast dynamics sample alternately their different behavioral modes. Our results are presented with compact, graphical descriptions which allow insightful interpretations for various physiological parameter dependencies and which summarize clearly the underlying mathematical structures. (Rinzel)

We have extended our fast/slow analysis (see "Bursting" above) to modified versions of the Chay-Keizer mathematical model for electrical bursting activity of pancreatic β-cells. These models involve a single slow variable, intracellular free calcium concentration (denoted as Ca). Previously, we have shown that the fast, spike-generating, membrane dynamics exhibit bistability over a range of Ca values: membrane potential might be either oscillating or at steady state. Our analysis shows why this bistability leads to an average membrane potential time course which appears more like a square wave than the sinusoidal-like slow wave of the Aplysia R-15 neuron (see below). This bistable behavior has also been explored and related to the pseudo steady-state current-voltage characteristics of the membrane. Recently, we have also compared different hypotheses for glucose dependence which predict differences in the range, i.e. maximum and minimum, of Ca (and in the time average of Ca) during bursting. If Ca-removal rate is treated as the glucose sensitive parameter, then the range is independent of glucose and the average Ca varies modestly. In contrast, both of these measures of the Ca response show significant

variation when increasing glucose is modeled by decreasing the conductance of a potassium channel (to represent the, recently discovered, ATP-sensitive potassium channel). Our theoretical results should further provoke experiments to measure intracellular calcium activity during bursting. (Rinzel, Atwater, and Chay)

The R-15 neuron of the Aplysia abdominal ganglion is a well studied experimental model for neuronal bursting activity. Under certain experimental conditions, an underlying, nearly sinusoidal, slow wave (without spikes) has been observed. We have applied our method of fast/slow analysis (see "Bursting" above) to elucidate the origin of bursting and slow wave behavior in a model for the R-15 oscillations. In this case, there are two slow variables: intracellular free calcium concentration (Ca) and a slowly activating membrane conductance (x) for calcium. We show that the dynamics of x and Ca, with the fast variables at steady state, are sufficient to generate a slow wave. In other parameter regimes, the slow trajectory carries the fast variables above the threshold for repetitive spike activity and bursting occurs. Theoretical burst patterns may exhibit the parabolic nature (i.e., low spike freqency at the beginning and end of a burst) of those seen experimentally and our analysis reveals the reason for this behavior. In the original model, a calcium-activated potassium-channel causes the repolarization following a burst. Our fast-slow analysis clearly reveals how, a recently proposed, alternative mechanism, calcium-inactivation of the slow calcium channel, can also account for the burst pattern. (Rinzel and Lee)

We consider a space-clamp experiment in which an applied current is turned on at t = 0 then slowly increased. Under such conditions, many nerves, especially those with a sensory function, would be expected to fire repetitively for values of applied current I in a certain interval but not for I too small or too large. Jakobsson and Guttman (1980) made an interesting experimental observation when investigating whether or not squid axons in low divalent cations accomodate to slowly rising currents. They found that a small but definite tendency for the repetitive firing to start at a lower current value when the current is applied more slowly. This counter-intuitive result showed that there is not just a lack of accomodation but in fact its opposite; the threshold apparently gets lower as the stimulus is maintained. Numerical simulations of Hodgkin-Huxley axons agreed with the experimental observations, and the magnitude of the effect was about the same. In some biological systems the control parameters change naturally. For example, in enzymatic reactions developmental transitions in Dictostelium Discoideum are related to a change in catalytic activities of two key enzymes. In this study we analyze nerve models where a slowly-varying bifurcation parameter is introduced as a new control mechanism. In particular, we study analytically and numerically the slow passage of this parameter through a Hopf bifurcation point using FitzHugh-Nagumo and Hodgkin-Huxley models. We find that for small ramp speeds the threshold voltage for repetitive firing is particularly sensitive to noise. (Baer, Erneux, and Rinzel)

Subcritical bifurcation is an important qualitative feature of the nonlinear response in Hodgkin-Huxley and Hodgkin-Huxley like nerve models. The general phenomena of subcritical bifurcation was first discovered by von Karman and his colleagues in 1935 while investigating elastic shell buckling. In nerve, the phenomena may be described as follows: Suppose an applied current is turned on at t = 0 and brought to a subthreshold value so that a steady voltage is reached and repetitive firing is not initiated. If the system is then perturbed by an "infinitesimal" disturbance the response will be a small amplitude damped oscillation (This damped oscillation is known to physiologists as subthreshold oscillations.). However, if a "finite amplitude" disturbance with proper magnitude is present the

system may destabilize and large amplitude repetitive firing results. The bistable nature of these dynamics is also necessary to explain the annihilation experiments seen in the nerve conduction literature. Using the asymptotic methods of Reiss and Tu (1986) we investigate the dynamics of the subcritical transitions from steady to stable periodic solutions for the space-clamped FitzHugh-Nagumo equation. We first consider steady and then periodic perturbation near resonance. (Baer and Mejia)

Last year we developed new perturbation methods to study singular Hopf bifurcation problems arising from relaxation oscillations characterized by two disparate time-scales (as may occur in some nerve membrane models). We continue the mathematical study of the relevant amplitude equation when it is weakly perturbed with periodic forcing. The goal to investigate analytically the properties of the domain of periodic solutions which pave the way to chaos. We construct, by a regular perturbation analysis, harmonic and subharmonic large amplitude periodic solutions, which may coexist. We then determine a small amplitude periodic solution oscillating at the forcing frequency and show that perturbations of this basic state decay except near certain critical points where further (period-doubling) bifurcations may occur. We have applied our results to study laser rate equations. (Baer, Erneux, and Mandel)

Synaptic Neurobiology

Excitable dendritic spine clusters: nonlinear synaptic processing. Background on both passive and excitable dendritic spine computations was provided in last year's report. This year our focus has been on exploring important implications of excitable spine clusters. The following computed examples illustrate some of the phenomena. For an extensively branched dendritic tree we assume 50 spines per dendritic branch. Usually only 5 spines per branch were given excitable spine head membrane. Because of large input resistance values at distal dendritic branch locations, the firing of only 2 excitable spines (by synchronous synaptic input) can produce enough local membrane depolarization to fire the other 3 excitable spines on that branch. This cluster of 5 can be extended to the firing of clusters of 10 or 15 spines on 2 or 3 adjacent branches by adding synaptic input to 2 or 3 passive spines; also cluster firing can be prevented by synaptic inhibitory input to only 1 or 2 excitable spines. Whether a cluster fires depends (with nonlinear sensitivity) upon changes in synaptic excitation or inhibition and upon changes of spine stem resistance (a possible locus for plasticity related to conditioning and/or learning) or of other spine parameters. These and other computed examples indicate the rich repertoire of logical operations that could be implemented by excitable spine clusters. (Rall and Segev)

Work has continued on biophysical theories for dendritic spine function. The recent focus has been to explore interactions between spines. For example, how excitation of a few spines, which receive synaptic input, may propagate to other spines, and perhaps into other dendritic branches. To facilitate analysis of interactions between many spines (distributed with high density over the dendritic tree), we have formulated a new cable theory in which the distribution of spines is treated by a continuum rather than discrete approach. For idealized but nonlinear membrane properties for the spine heads, we have characterized analytically various parameter domains for which propagation along a single branch is, or is not, possible. To explore continuum spine models with multiple dendritic branches, and with less idealized membrane properties, new computational tools continue to be developed. We are currently investigating threshold properties (e.g., minimum number of synaptically activated spines in one or more branches) necessary for propagation, or

significant amplification of the dendritic response. Our results complement the discrete spine simulations of Rall and Segev. (Rinzel and Baer)

Non-uniform Rm in Dendrites. The membrane resistance, Rm, of dendritic membrane may be non-uniform due to different densities of ionic channels in different parts of the dendritic tree or non-uniform opening or closing of ionic channels due to non-uniform patterns of synaptic activity. The effects of non-uniform Rm on the effectiveness of individual synaptic inputs (as measured by the peak transient or steady-state potential change at the soma) and on some of the electrophysiological properties of a neuron were studied in models of cortical pyramidal cells. Significant changes in the effectiveness of distal inputs were found with different Rm distributions suggesting that distal inputs can be effective or ineffective in producing a change in soma potential depending on the activity of other inputs in the dendritic tree. It should be noted that changes in Rm distributions may occur when afferents are cut in the in vitro tissue slice preparation and these changes can affect the values of experimental electrophysiological parameter estimates and the effectiveness of individual inputs. (Holmes and Woody)

It was shown that applying methods for calculating the electrotonic length (L) of a neuron based on a uniform equivalent cylinder can give misleading results when applied to neurons with non-uniform Rm (for which the formula is not valid, but often used). A new method for calculating L was discovered which appears to be more robust to deviations from equivalent cylinder assumptions about branch dimensions. This method still assumes a uniform Rm can be easily adapted for the case where there is a step change in Rm. (Holmes and Rall)

Optimal weighting of synaptic inputs. Given the length of each dendrite in a dendritic tree along with the electrophysiological parameters Rm and Ri, the effectiveness of a given synaptic input (as measured by the peak transient or steady-state potential change at the soma) is maximized for a set (non-unique) of diameters of the dendritic processes. Conditions for optimal effectiveness have been explored analytically for the steady-state situation in simple cases. The existence of optimal dendritic diameters has been demonstrated numerically in a model of a cortical pyramidal cell. The dendritic diameters determine the locations of inputs (depending upon synaptic conductance magnitude) which are operating at maximal effectiveness and this has significance for how inputs are weighted in synaptic integration. (Holmes and Rall)

Molecular Biology

We are refining previously developed algorithms for sequence comparisons. Our previous methods rapidly scanned the two dimensional matrix identifying subsequence alignments allowing mismatches but no gaps for an initial similarity score, and then performing a complete optimization in a band encompassing the most similar segment. The new methods perform an optimization of the subsequence alignments found in the initial scan to obtain an initial score which allows for insertions and deletions with virtually no increase in computation time. This algorithm has been implemented in nucleic acid and protein search programs. Empirical tests of these programs have detected a number of significant relationships that would not be detected by the previous method. (Lipman and Pearson)

The construction of multiple alignments (consensus sequences) of proteins can often reveal important aspects of the evolutionary and functional relationships in a protein family that will not be seen using pairwise alignments. A well defined

automatic method which produces biologically relevant results is not yet available. Simple generalizations of methods used for pairwise alignments are not computationally feasible. We are developing a method for the simultaneous alignment of up to 5 amino acid sequences. We decrease the computational complexity of the task by first finding statistically significant pairwise homologies and then obtaining an optimal or near optimal alignment of the remaining sequence using a modification of a recently developed optimization algorithm. (Lipman and Polner)

Efforts to inform and educate molecular biologists about the new computational tools for sequence analysis have continued. A heavily attended "hands on" workshop was put on in the fall using the Computer Users Resource Center.

We have analyzed the variation in the solvent accessibility and hydrophobicity of the amino acids along the sequences of 58 soluble globular proteins with known tertiary structure. We find a significant tendency for clustering of accessibilities along the sequence but that hydrophobicities are distributed randomly. These results suggest severe limitations on the power of sequence analysis tools which use average hydrophobicity scores to predict solvent accessibilities. Furthermore, we show that this result is not directly related to secondary structure. (Lipman, Lee, Pastor)

Because of new rapid sequencing methods, we now have a sizable number of related amino acid sequences for many different protein families. We are interested in developing quantitative methods for using this information on protein families. One project involves developing more sophisticated sequence similarity measures which are position specific based on the pattern of sequence conservation in a protein family. For example, to test whether an unknown protein is a member of a family, one would compare against a consensus sequence, some of whose positions would be "less important" (i.e., have a lower weight) than others, based on relative conservation. Preliminary analyses are promising, in that one can detect an increased difference between the expected score and the observed score for distant relationships. This is complicated by a concomittant increase in variance of the expected similarity score. Another approach is to recode the sequence of amino acids in terms of quantitative properties such as hydrophobicity, bulk and polarity. For any position in a family consensus sequence one would compute the mean value for each property and an associated weight related to the variance of that property (i.e., if a position strictly conserves an intermediate bulkiness for the amino acids at a position, that value would be weighted highly). For some position only size may be important, for others, polarity and bulk. This new consensus based on physical properties may then be used to detect weak sequence, structure similarities, or to detect the minimal sequence differences necessary for specific functional differences. (Lipman)

We have developed a general, mathematically rigorous algorithm for sequence comparisons which takes advantage of sparse matrices. The method is related to earlier computer science methods for finding the longest common subsequence, but allows for gap penalties. In the average case (two unrelated sequences), it will find the maximal subsequence based on scores for matches and penalties for gaps in time proportional to the number of matches between the two sequences. We are currently evaluating appropriate applications of the method to biological sequence comparisons. (Lipman)

Analysis has continued of the PAM matrix model (M. Dayhoff, et al.) of protein evolution by point mutations. The PAM matrix model predicts a quadratic dependence of the number of doubly replaced amino acids on the divergence time. An investigation is under way to determine how well available sequence data agree with such a quadratic dependence. (Wilbur)

It is widely held that most mutations of genetic material are slightly negative in their effect upon the organism. This raises the question of how mutations may influence the overall fitness of a population. A model studied by Movan has been found useful in studying the question. Effects are found to be significant for infinite populations but become quite marked for small populations. It is predicted that small populations will experience a systematic deterioration in their fitness which is distinct from genetic drift. (Wilbur)

Microcirculation and Facilitated Transport

The formulation of the dependence of the facilitated transport of oxygen on the substrate concentration at the boundaries of the transport path has been described in a previous report. The carrier is taken to be myoglobin. The results discussed there have led to the formulation of a global control principle of the facilitated transport and this principle has been illustrated for the physiological parameters that pertain to the Ascaris lumbricoides and for vertebrate striated muscle.

The concepts referred to above originate on the observation that for each fixed O_2 concentration at the low O_2 concentration boundary of a transport path the facilitated transport first increases and then decreases as the O_2 concentration increases at the high O_2 concentration boundary. Our theoretical results agree with experimental results of Wittenberg in which the O_2 concentration at the high concentration boundary was changed.

Further we have tried to identify the physicochemical entities of the transport process involved in this behavior so that we may have a clearer description of the phenomena in terms of the biological important parameters of intracellular fluxes.

The existence of a larger flow of substrate when the carrier is present depends on the feature that inside the transport path the total flow F is shared between the flow of the dissolved substrate F_d and the flow of the substrate combined with the carrier F_c. When the substrate concentration at the high concentration boundary is increased within some limits the increment of the value of F_c is associated with the increment of the facilitated flow. However, we showed that beyond some value of the boundary concentration a further increment of F_c accompanies a decrease in the facilitated flow. This phenomenon depends on the nonlinearity of the chemical kinetics of the carrier-substrate association. It does not take place for the linear chemical kinetics case. In terms of flows of free substrate F_d and of substrate-carrier compound F_c inside the transport path the mechanism involved appears to be the following. Inside the path, and away from the boundaries, the flow of free substrate is transferred to the flow of combined substrate. Then at the low concentration boundary the reverse process takes place. The latter necessitates an increase in the concentration of the combined substrate at the low concentration boundary and since by the nonlinearity of the process the concentration at the high concentration boundary is unchanged then the facilitated transport decreases.

Another feature studied was the dependence of the facilitated transport on the length of the transport path. When the substrate concentration at the boundaries are fixed but the length of the transport path is decreased the facilitated transport, in general, first increases and then decreases. This occurs in both linear and nonlinear chemical kinetics cases.

However, for the nonlinear case a biphasic behavior may appear: as the length of the transport path decreases the facilitated transport experiences two maxima before tending to zero. This phenomena was pointed out originally by Kreuzer and Hoofd but no explanation of its mechanism was offered. We found that this biphasic behavior depends on changes on the transfer of flows that take place only inside the solution and away from the boundaries. Our results also point out a limitation on the use of the Damkohler number (a parameter sometimes used to distinguish transport behavior which is flow limited or chemical kinetics limited) to characterize global changes of the facilitated transport. According to the usual definition, the Damkohler number changes monotonically as the length of the transport path changes, but our results show that the facilitated flow may change biphasically and exhibit two maxima.

A further application of the analysis of the distribution of free and combined oxygen flows, along the transport path was to the study of the symbiotic association between some plants carrying hemoglobin in their cytoplasm (legumes, Parasponia) and nitrogen fixing bacteria (Rhizobium) present inside the nodules of these plants. On the one hand the enzyme nitrogenase, that converts N_2 to ammonia, is destroyed by oxygen in small concentrations but on the other hand the nitrogenase activity in the bacteroids depends on the supply of ATP formed by bacterial oxidative phosphorylation. "The paradox of root nodule function is how a large influx of oxygen is sustained, while the O_2 concentration within the bacteroid is kept at a very low level and the nitrogenase activity unimpaired" (Wittenberg, Appleby). Also in these cases, large gradients of oxygen concentration near the oxygen entry site, and low flat profiles of oxygen concentration in the rest of the cell have been found.

By using the information on the chemical kinetics of the association of Parasponia Hb and leghemoglobin with O_2 and on the other parameters that correspond to the plant nodules that host the bacteroid symbiont Rhizobium, the carrier transport equations were numerically solved. The results show a large gradient of free oxygen in an interval close to the high oxygen concentration boundary and a very flat and low concentration for the rest of the transport path. The results support the notion that these plant hemoglobins are capable of effecting a large influx of oxygen to the bacteroids and yet maintaining a low concentration of free oxygen. The transport of oxygen would be mediated through the compound oxygen-hemoglobin and not through the free oxygen. (Gonzalez-Fernandez)

Auditory Physiology

A minimal model has been developed which accounts qualitatively for various features of mammalian cochlear processing at low frequencies typical of human speech. Parameter sensitivity of the model has been carefully explored. The model is most sensitive to basilar membrane width and thickness. While not particularly sensitive to viscosity in the physiological range, the response changes markedly if viscosity is removed from the model. However, many combinations of parameters produce reasonable results, making careful measurements of cochlear properties very important for prediction of physiological parameters. Further, this suggests that the tonotopic map is learned rather than hard-wired in the brain from birth. The model has also been used to investigate responses to complex sound stimuli. In particular, it

accounts for synchrony suppression phenomena as well as other nonlinear effects observed in the synchronous components of the responses, and enables an evaluation of their relevance in the encoding of speech sounds. Finally, spatio-temporal response patterns on the auditory nerve to natural speech stimuli have been simulated. These patterns have been analyzed by a variety of neural network models to extract signal parameters and to mimic the recognition process that the CNS performs when identifying these stimuli. A number of learning algorithms are being explored to compute the pattern of connections that these networks must possess in order to perform their specific tasks. (Shamma, Chadwick, Morrish, and Rinzel)

Analysis for Processing of Data from NMR, Ultrasound, and Radiological Imaging

An ultrasound study of speech and swallowing continues. Recent work explores location and amount of maximum displacement and maximum curvature of the midsagittal tongue during nonstressed production of five different vowels across normal subjects. It can be shown that individual variation is larger in anterior-posterior position than in height, consistent with anatomical constraints. In addition, amount of displacement at maximum distinguishes between all vowels but /o/ and /æ/ in all subjects, and exhibits the same pattern in all subjects, suggesting a normal standard. This is not the case for curvature, implying that perhaps a number of oral configurations can produce a recognizable vowel. (Morrish, Stone, Sonies, Shawker, and Baum)

A radiological study of metacarpophalangeal (MCP) patterns in patients with Prader-Willi syndrome (PW) has been completed. Statistical analysis has shown that (i) the (MCP) bones continue to fall behind in size relative to those of normal subjects throughout childhood, (ii) the MCP bones are shorter in PW children than in normal children, (iii) canonical variables presented in the literature as candidates for diagnostic parameters by which to distinguish the two subgroups of PW patients and PW patients from normals did not do so in the present data. Because of the inhomogeneity in the effect of PW syndrome on MCP bones, measurements based on the bones provide unreliable clinical tests for PW syndrome. (Morrish, Nagele, Ryan, Sidbury and Doppman)

A two-part study of imaging methods for parathyroid adenomas has been undertaken. The first part involves noninvasive techniques, including ultrasound (U), computed tomography (CT) and Technetium/Thallium Scintigraphy (S). Statistical analysis has shown that (i) U performs better in the neck than the chest, and (ii) when performing multiple tests for diagnostic purposes, the order in which to test that has the highest probability of yielding two positive tests in patients with an adenoma places U and CT first, followed by S. The second part examines invasive imaging methods: digital angiography (D), conventional angiography, venous sampling (V) and intraoperative ultrasound. It was shown that V performed significantly better than D; however, since all techniques could identify between roughly 50 and 80 percent of the adenomas, their use can be based largely upon convenience, cost and patient comfort and safety. (Morrish, Miller, Doppman, Shawker, Krudy, Vucich, Norton, Marx, Spiegel and Aurbach)

It is generally accepted now that an undistorted spectrum can be obtained by either cross correlating the rapid scan responses of the unknown and a reference consisting of only a single sharp NMR line or, alternatively, by applying to the Fourier transformed response an appropriate analytic function. It has also been shown that

rapid scan FT-NMR compares favorably with pulse FT methods in sensitivity and has some distinct advantages over the pulse technique. We have conducted theoretical NMR experiments, consisting of solution of the Bloch equations for varying sweep rates, spectral decomposition and filtering, that have shown a broadened signal peak for order of magnitude increases in sweep rate. The objective of our investigation is to reduce the sampling time required for an experiment. (Ferretti, Mejia, and Weiss).

Renal Physiology

Theoretical work continues on proton, bicarbonate and ammonia transport in the mammalian proximal tubule and cortical collecting duct (as described in previous year's report). A mathematical model of a perfused renal tubule in a bath that describes concentration profiles for total Co_2 (i.e. Co_2, HCO_3^- and H_2CO_3) and H^+ concentration in two space dimensions has now been extended to include total ammonia and a third buffer. Computer based experiments to date have shown that (1) a physiological rate of proton secretion can generate substantial radial gradients $[H^+]$ and $[H_2Co_3]$ in the absence of luminal carbonic anhydrase, (2) an uncatalyzed reaction (dehydration rate constant of 49 s^{-1} for carbonic acid) is consistent with NH_3 permeability measured experimentally, (3) the concentration or pK of a third buffer, such as phosphate or HEPES, can modify the concentration gradients significantly. (Mejia, Star, and Knepper).

In the theory of acid base balance, controversies concerning mechanisms of pH regulation are hinged in part on a lack of agreement about what acid-base balance is. We are in the process of developing a rigorous description of pH balance that is based on physical principles. The hydrogen ion concentration of a control volume is determined by proton balance, where a control volume is any geometrically closed space with definable inputs and outputs (e.g. a beaker, a cell, extracellular space, a single tubule segment, a kidney, etc.). Any general theory of proton balance should apply to any such control volume. Hence, the hydrogen ion concentration of a control volume must satisfy mass, volume and charge conservation equations, including electroneutrality. The model of a perfused tubule described previously is an example of an application of these basic principles. We plan to apply it to other control volumes. (Knepper and Mejia).

A two-dimensional mathematical model of a radially symmetric slow flow system in a long cylinder with moderate wall leakage and physical parameters of the renal proximal tubule has been developed. A study involving multiple solutes is in progress, as well as a closer look at the behavior of the physical system at the entrance and exit of the tubule. In particular, relatively high radial velocities are expected at the axial boundaries. (Morrish and Kellogg)

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 13,001-13 MRB
Formerly
Z01 AM 13,001-12 MRB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mathematical formulations and analysis relevant to experimental neurophysiology.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W. Rall	Senior Research Physicist	MRB, NIDDK
Others:	W. R. Holmes	NRSA Fellow	MRB, NIDDK

COOPERATING UNITS *(if any)* Lab. of Neural Control, NINCDS
Dept. of Zoology, Univ. of California, Berkeley
Dept. of Anatomy, Yale University
Dept. of Neuroscience, The Hebrew Univ. of Jerusalem

LAB/BRANCH
Mathematical Research Branch

SECTION

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.3	2.0	.3

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This research involves creating, exploring and testing mathematical models of dendritic neurons that are relevant to experimental neurophysiology and neuroanatomy. Together these models provide a theory that can account for various sequences of events in the soma and dendritic branches of a single neuron, and for field potentials generated by certain cortical populations of neurons. Computational experiments performed with these models provide theoretical predictions that have been compared with experimental results obtained by colleagues with motoneurons of cat spinal cord, and with the mitral cell and granule cell populations of rabbit olfactory bulb. Resulting interpretations contribute to understanding of dendritic synaptic input and of dendro-dendritic synaptic interactions. Some of these results are summarized in Chapter 3 of "The Handbook of Physiology: The Nervous System, Vol. 1", American Physiological Society (1977).

Several important consequences of assuming excitable membrane properties at the heads of dendritic spines have been explored computationally and presented at symposia. For distal dendritic locations, the firing of one (or a few) excitable spines may trigger (through spread of sufficient depolarization along the dendritic shaft) the firing of neighboring excitable spines. Such a chain-reaction will usually spread (and fire) only a subset of the available excitable spine clusters. Whether a spine cluster fires or not depends (with nonlinear sensitivity) upon changes in synaptic excitation and inhibition and changes in spine stem resistance or other spine parameters. The thousands of spines and synaptic contacts per neuron can provide a rich repertoire of logical operations implemented by excitable spine clusters; explicit examples are being explored.

The collaboration of Holmes and Woody extends similar neural modeling and testing to include complications resulting from non-uniform R_m and non-uniform synaptic background activity in pyramidal cells of cerebral cortex; explicit examples are being explored.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 13,002-14 MRB
Formerly
Z01 AM 13,002-13 MRB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mathematical description of substrate transport in capillary-tissue structures.

PRINCIPAL INVESTIGATOR *(List other profassional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J. M. Gonzalez-Fernandez Research Mathematician MRB, NIDDK

COOPERATING UNITS *(if any)*

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Mathematical Research Branch

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INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.03	1.0	.03

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goal of this work is to develop mathematical models of the blood flow and transcapillary exchanges in capillary networks. An effort is being made to incorporate in the models the histological structure of capillary networks as well as different flow patterns from available experimental information. In this model the extraction of substrates with different chemical kinetics at the tissue site will be described. It is expected that this could be used in experimental situations where the extraction of different substrates are measured simultaneously, thus helping to infer the flow pattern features of the microcirculation. In particular a model of the diffusion-consumption of oxygen in striated muscle containing myoglobin (facilitated diffusion) is being developed and pertinent numerical results examined.

A possible adaptive mechanism for the facilitated transport of oxygen by hemoglobin in nematodes (Ascaris lumbricoides) and myoglobin in vertebrate striated muscle has been investigated.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 13,004-12 MRB
Formerly
Z01 AM 13,004-11 MRB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mathematical description of cellular neuroelectric signal transmission.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. Rinzel	Chief, MRB	MRB, NIDDK
Others:	S. A. Shamma	Guest Worker	MRB, NIDDK
	Y. S. Lee	Visiting Fellow	MRB, NIDDK
	K. A. Morrish	Staff Fellow	MRB, NIDDK
	S. M. Baer	Staff Fellow	MRB, NIDDK

COOPERATING UNITS *(if any)*
Lab. of Cell Biology & Genetics, NIDDK
Biomedical Engineering & Instrumentation Br., DRS
Dept. of Biological Sciences, Univ. of Pittsburgh

LAB/BRANCH
Mathematical Research Branch

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INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.9	2.5	.4

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project continues to focus on the formulation, analysis, and biophysical interpretation of mathematical models which describe various aspects of neuroelectric signaling for individual neurons. Among the topics of current interest are: (i) integration of synaptic input delivered to the soma and dendritic branches of a neuron; (ii) propagation of action potentials along axons; (iii) stimulus-response and threshold properties for repetitive-firing of action potentials; (iv) complex bursting patterns of membrane potential oscillations which arise through endogenous membrane properties and/or interneuronal coupling.

Because qualitatively related mathematical or biophysical problems may arise in other contexts, e.g. chemical and biochemical oscillations, or e.g. excitation-secretion coupling, this project may consider models from such applications.

Mathematical models of these phenomena involve systems of linear and nonlinear ordinary differential equations and parabolic partial differential equations. Solutions and their mathematical stability are determined by analytical and numerical methods drawn from both classical and modern applied mathematics. These methods may include finite difference or finite element numerical integration, bifurcation theory, perturbation techniques, and nonlinear dynamical systems theory. One goal of this project is to expose the qualitative mathematical structure for classes of models by exploiting simple, yet physiologically reasonable, equations.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 13,014-05 MRB
Formerly
Z01 AM 13,014-04 MRB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Probabilistic Analyses of Nucleic Acid Sequences.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. J. Lipman	Research Scientist	MRB, NIDDK
Other:	G. P. Polner	Visiting Fellow	MRB, NIDDK

COOPERATING UNITS *(if any)*

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INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.44	1.4	.04

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has focussed on the analysis of amino acid and nucleic acid sequence data as it pertains to molecular biology and molecular evolution. Continuing areas of interest include:

i) The development of computational tools for molecular biologists. We have continued to refine earlier sequence comparison algorithms with significant improvements in sensitivity. We are also developing a method for the simultaneous alignment of up to five amino acid sequences.

ii) We have analyzed the variation in the solvent accessibility and hydrophobicity of the amino acids along the sequences of 58 soluble globular proteins with known tertiary structure. We find a significant tendency for clustering of accessibilities along the sequence but that hydrophobicities are distributed randomly. These results suggest severe limitations on the power of sequence analysis tools which use average hydrophobicity scores to predict solvent accessibilities.

iii) We are studying the patterns of sequence conservation in protein families to develop improved methods of detecting evolutionary relationships, structural similarities, as well as the minimal differences necessary for specific functional differences.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 13,015-05 MRB
Formerly
Z01 AM 13,015-04 MRB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Probabilistic modeling of biological information systems.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W. J. Wilbur	Guest Worker	MRB, NIDDK
Others:	J. Rinzel	Chief, MRB	MRB, NIDDK
	D. J. Lipman	Research Scientist	MRB, NIDDK
	S. A. Shamma	Guest Worker	MRB, NIDDK
	R. Chadwick	Biomedical Engineer	BEI, DRS

COOPERATING UNITS *(if any)*

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INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.3	.25	.05

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Work has been carried forward in three different areas. A model of cochlear processing of auditory stimuli has been developed. Hair cell transduction is the only nonlinear component in the model and this suffices to explain well known nonlinear auditory characteristics. The second area involves a study of cellular automata. Cellular automata provide a unique approach to complex biological problems such as growth and differentiation of organisms and neural structure and function. Much current interest focuses on general properties or approaches to cellular automata. We have developed an analytical procedure for predicting the statistical distribution of local patterns in a class of simple automatons. The method gives promise of generalization to more complex systems. The third area of study is the statistical properties of biological macromolecules. The PAM matrix model of protein evolution has been analyzed and an error discovered in the construction of the matrix and a need to modify the model demonstrated. A study is currently in progress to determine the effect of mutations on the overall fitness of a population. Preliminary results suggest a large effect for small populations.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 13,017-03 MRB
Formerly
Z01 AM 13,017-02 MRB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Sound processing in the auditory system.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S. A. Shamma	Guest Worker	MRB, NIDDK
Others:	J. Rinzel	Chief, MRB	MRB, NIDDK
	R. Chadwick	Biomedical Engineer	BEI, DRS
	K. A. Morrish	Staff Fellow	MRB, NIDDK

COOPERATING UNITS *(if any)*
Biomedical Engineering & Instrumentation Br., DRS

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NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.2	.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

These projects have considered the processing of sound at various stages in the auditory system. These studies include:

(1) Theoretical models of the peripheral stages of the system. The computed results agree well with experimental findings and help explain several observed nonlinear phenomena.

(2) The development of realistic algorithms (as model neural networks) to process speech evoked activity on the auditory nerve. The results point to new ways of viewing the auditory nerve code.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 13,018-02 MRB
Formerly
Z01 AM 13,018-01 MRB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 chererters or less. Title must fit on one line between the borders.)*
Mathematical analysis of biomedical systems.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	K. A. Morrish	Staff Fellow	MRB, NIDDK
Others:	J. Rinzel	Chief, MRB	MRB, NIDDK
	S. A. Shamma	Guest Worker	MRB, NIDDK

COOPERATING UNITS *(if any)* Inst./Physical Sci. & Tech., U./Maryland, College Park; Dept. Radiology, CC; Dept. Rehab. Med, CC; Biomed. Eng. & Instru., DRS: Clin. Invest. & Patient Care, NIDR; Div. Cancer Treat., NCI; Metabolic Dis., NIDDK; Lab. Cell Biol. & Gen., NIDDK.

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INSTITUTE AND LOCATION
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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.2	3.8	.4

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the spece provided.)*

This project involves biomedical research at levels from experiment design to the biophysical interpretation of mathematical models. When data cannot be obtained from the literature, an experimental protocol must be initiated. The design of such a protocol involves identifying quantities of interest, promising measurement techniques, and plans of action which should yield results which can be evaluated for reliability and significance using applicable statistical techniques. Once good data have been obtained, the process of mathematical modeling proceeds. This may involve differential equations which describe changes in the system over space or time, such as the Navier-Stokes equations, and fits to experimental data. Complicated systems are often simplified by techniques such as dimensional analysis or WKB methods. The resulting simplified equations may be amenable to analytical techniques which yield information on properties of the solutions, such as existence and uniqueness. Numerical techniques such as finite differences are usually employed to solve the systems. The development is designed with an eye toward interpretation of the results, for it is at this point that the descriptive and predictive powers of the model are revealed.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 13,019-02 MRB
Formerly
Z01 AM 13,019-01 MRB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mathematical study of excitability properties in coupled nerve membrane patches.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: S. M. Baer Staff Fellow MRB, NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Mathematical Research Branch

SECTION

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.02	1.0	.02

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Biophysical theory regards the electrophysiological interaction between passive and active patches of nerve membrane as functionally significant. Important examples are interactions such as an active soma attached to a dendritic tree, myelinated nerves, and the possibility of dendritic spines with excitable spine head membranes. The aim of this project is to explore, using mathematical modeling, analysis, and numerical computation the functional implications of these interactions.

We expand this study to include a new class of excitability problems involving a slowly-varying control parameter and we also continue to study the voltage response of nerve membrane when subjected to periodic forcing.

PHS 6040 (Rev. 1/84) GPO 914-918

ANNUAL REPORT OF THE LABORATORY OF CELLULAR AND DEVELOPMENTAL BIOLOGY

NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

This laboratory supports fundamental research in areas which are at the forefront of modern cell biology and biochemistry. The realization that many facets of research which have led to discoveries of potential major impact in health care have derived from basic scientific investigation in the past decade lends credence to such efforts at the National Institutes of Health, in general, and in the LCDB, in particular.

The spectrum of investigations within the laboratory is quite broad but the various areas are intertwined in a way which allows interactions between different working groups for the benefit of all. There is a logical thread from structural studies of chromatin, viruses, proteins and cytoskeleton to investigations of genes and factors during development and differentiation to the role of cyclic nucleotides and hormones in cellular metabolism to studies of the physiology of hormone responsive cells, particularly adipocytes, to lipid transport and metabolism to enzymology and finally to applied biology. As in the past, to emphasize this continuity within seeming diversity, we will review the work of the laboratory by topical areas and not by individual working groups. We will follow the road outlined above in this report of LCDB's activities in the past year.

High resolution structural studies

The Structural Biology Section is a new addition to LCDB this year. Using high resolution electron microscopy and computer-based image enhancement methods, this group provides a pleasant complement to the more biochemical/molecular biological approaches of other parts of the laboratory. A number of experimental systems are under active investigation in collaboration with investigators at the NIH and elsewhere. Using electron microscopic analysis of paracrystalline arrays, the structure of the Bordetella pertussis fimbriae has been determined to be a single start, 2.5 subunit per turn, 7 nm diameter helix. This is unlike the structure of other bacterial fimbriae previously determined.

Several viral structures are subjects of investigation. 1) Localization of M protein of vesicular stomatitis virus, a major protein that condenses the nucleocapsid into a helical coil, has been attempted by image analysis of various forms of the virion. Current interpretation of the data suggests a localization between the nucleocapsid and the viral membrane; this is somewhat uncertain due to technical inconsistencies between negatively stained and frozen dried samples. 2) Six filamentous tail fibers of bacteriophage T7 are composed of protein gp17. These fibers are thought to recognize surface

receptors on susceptible bacteria. The structure of the fiber appears to be a trimer of gp17, organized in three domains, an amino terminal region which links fiber to tail, a central coiled coil helical segment, and a carboxyl terminal nodular globular section. 3) the gp23 surface lattice protein of bacteriophage T4 capsid is an interesting molecule which undergoes an ordered series of conformational changes during phage head assembly. These have been characterized by differential scanning calorimetry and we have suggested that specific transitions represent separate denaturations of species of unexpanded particles that have not been distinguished by morphological techniques. Using the sequence of gp23, we have identified likely epitopes and elicited antibodies to peptides synthesized to match same. These are now being used in immunomorphological studies to map the location of the epitopes in the various conformational states.

Organization of two cytoplasmic protein families is the subject of continuing investigation. Computational techniques have been brought to bear on the structure of intermediate filaments, one of the three major components of the cytoskeleton; the goal is to resolve controversy concerning the axial repeat of the helical rod domain in the filaments. Results suggest that the repeat is conserved in epidermal keratin intermediate filaments containing subunits of differing molecular weight, consistent with a generic model for structure of these filaments previously proposed by us. We have begun studies of vertebrate skeletal muscle in different physiological states using frozen-hydrated thin sections to complement previously reported conventional thin section electron microscopy and x-ray fiber diffraction. Technical difficulties in such studies have been resolved to a large extent by development of computer software to handle the problems; initial results establish a definite difference in myofilament unit cell structure between relaxed and rigor states, consistent with attachment of myosin S-1 to actin filaments in the rigor state.

The structure of rat liver coated vesicles has been addressed using STEM, image processing and biochemical compositional determinations. About 30% of isolated "coated vesicles" do not contain vesicles at all; rather they appear to have a protein core, perhaps clathrin. The remaining members of the vesicle preparation are quite heterogeneous in size; in addition to lipid and clathrin, they contain a very diverse collection of proteins which comprise about 25% of the mass of the particle.

Two other areas of research in the laboratory are involved with atomic level structural studies. For many years, an interest of the laboratory has been the structure of barnase, an extracellular ribonuclease of Bacillus amyloliquefaciens, and barstar, an intracellular inhibitor of the enzyme. The proteins form a 1:1 inactive complex and both have simple two-state thermal transitions, making them an ideal system for study of protein folding and protein-protein interactions. The sequence and crystal structure of barnase have been determined previously and last year we reported the cloning of the gene for barnase using transposon TN917-mediated inactivation. We have now found, as previously suspected, that the complete gene for barnase is lethal when cloned in B. subtilis or E. coli. However, by using oligonucleotide directed muta-

genesis to replace HIS-102, an active site residue, with other amino acids, the gene can be expressed in both organisms. Processing of the preproteins is not correct in either case, however. To overcome this problem, all of the DNA 5' to the mature protein was replaced by the promoter and signal sequence of the E. coli phosphatase-A gene. With this construction, large amounts of authentic length (but inactive) barnase have been obtained from the periplasmic space of E. coli bearing the plasmid. More than 90% of the protein in the culture medium and periplasmic space is the mutant barnase. The ability to express in large amounts an inactive barnase, but one which folds correctly, will allow investigation of the mechanism of, and residues important in, folding of this interesting small protein.

In the past, studies of barstar or the complex of barstar with barnase have been difficult due to the minute amounts of the inhibitor normally produced. Recently, using a new method for generation of antibodies from small amounts of protein and a library of B. amyloliquefaciens DNA in λgt11, we have identified a clone which synthesizes this inhibitor. The gene product inhibits barnase, but not other ribonucleases, suggesting that the authentic barstar gene has been cloned. Western blots of the cellular extract of the plasmid bearing cells reveal two protein bands when reacted with anti-barstar; neither is the size of native barstar, suggesting that processing of the protein in E. coli is not correct. This should present only a small problem to production of large quantities of the protein for physical studies since a strategy similar to that employed for barnase production can be employed. Very recently, active barnase has been expressed in and exported from E. coli by cloning the barnase gene in a plasmid which also expresses barstar; this certainly confirms the identity of the barstar clone and the lethality of barnase in the absence of the inhibitor.

The second system which we have been able to study at this level of resolution is chromatin. Several years ago we described the formation of a positioned core particle when a cloned 5S rRNA gene was associated with chicken erythrocyte histones. Interactions of histones and DNA were extremely precise; we estimate that the position of the DNA on the histone octamer was localized with +/- one base pair. Later studies showed that the same positioning of a nucleosome occurred when the 5S DNA sequence was assembled into chromatin in vivo in yeast, suggesting that the positioning signal was quite general, if not universal.

In collaboration with Drs. Timothy Richmond and Aaron Klug of the MRC in England, this 5S gene has been developed as the first defined sequence DNA to be associated with histones and crystallized for a high resolution analysis of core particle structure. Minor modifications to the propositus DNA have been made to facilitate preparation of 146 bp DNA which is centered on the histone octamer. Association of this DNA with core histones and subsequent crystallization of the complex has led to material which diffracts to under 5 angstroms, a significant improvement in resolution over the earlier studies from the MRC group which utilized core particles isolated from native, and therefore random sequence, nucleosome core sources. Study of this homogeneous core particle in crystalline form should contribute strongly to resolution of the structure of the core particle and aid in resolving the current controversy about the structure of the histone octamer in the core particle vs in solutions of high salt concentrations.

Using polymeric constructions of DNA fragments based on the 5S ribosomal RNA sequence, we previously showed that positioned nucleosomes could form on linear, or relaxed or supercoiled circular, DNA. We have continued efforts to reassociate such multimeric fragments with not only core histones, but also lysine-rich histones of the H1 family. We have constructed multimers with repeat lengths of 180, 207 and 255 bp and isolated histones from rat liver, chicken erythrocyte, and sea urchin sperm (the lysine rich histones associated with chromatin having the above repeat lengths). Attempts to reconstruct higher order chromatin from the above reagents are in progress; a major problem in the reassociation is the difficulty in assessment of correct reconstitution. Should conditions be found which allow correct reconstitution of chromatin using defined DNA sequences, the resultant material should be amenable to high resolution solution and crystallographic analysis; this would provide a major advance in the solution of the structure of higher order chromatin, a level of structure which has been implicated in regulation of genomic transcription.

Chromatin structure and transcriptional regulation

We have described previously the alterations in chromatin structure that accompany activation of transcription of the sea urchin early histone genes at morula and reversal of these structural changes when the genes are rerepressed at blastula stage. We are interested in identifying the presumed trans-acting factor(s) that leads to either gene activation as a positive effector or repression as a negative one. We have utilized Western blots of low salt wash nuclear proteins probed with DNA segments that contain one of the nuclease hypersensitive site of the histone genes to try to identify such a factor. A protein of apparent molecular weight 55,000 binds the probe sequence tightly and specifically. This protein is present in extracts from morula nuclei but not in those from blastula nuclei, strengthening the possibility that it might be a specific regulatory factor and heightening our interest in its further purification and study.

While this approach to identification of trans-acting factors has been used by others, it suffers from the factor being identified only by its interactions with naked DNA, as opposed to with chromatin. We have begun another approach to study of transcriptional regulation which differs in kind from those utilized by others. We will isolate unique regulated genes, as chromatin, in different states of activity. The first phase of this study is now completed. We have previously extensively characterized the structure of a yeast "minichromosome", the 1453 base pair episomal TRP1ARS1 plasmid, and used it for investigation of nucleosome positioning mechanisms. Now, we have developed methods which allow purification of the amplified plasmid, as chromatin, to apparent biochemical homogeneity. Analyses of the kinetics of elution of the plasmid from nuclei and the properties of the non-eluted material suggest that the isolated material is representative of >90% of the total plasmid population. The plasmid, when eluted from nuclei, is transcriptionally active, an important observation for our eventual goals. The minichromosome contains

histones as its major proteins together with a restricted number of nonhistone proteins, some of which may be present in stoichiometric, i.e. one per plasmid, amounts. Linking number analyses and electron microscopic observations both suggest that the minichromosome contains seven nucleosomes, as predicted from our earlier nuclease cutting site mapping studies.

While effective, the purification procedure using conventional biochemical techniques is labor intensive and time consuming. We have attempted to use protein-DNA affinity to purify the minichromosome and preliminary results are quite enouraging. We inserted a segment of DNA containing the E. coli lac operator into an open, nucleosome-free, region of the plasmid DNA. Then, using a β-galactosidase-lac repressor fusion protein and anti-galactosidase antibody bound to beads, we can selectively remove the minichromosome from a nuclear eluate. Addition of an inducer of the lac operon, IPTG, releases the minichromosome from the beads. Initial analyses appear to demonstrate the integrity of the minchromosome after the isolation and suggest that it is at least 50% pure after this two step procedure. We are now in a position to insert into this vector regulated yeast genes and isolate such minichromosomes when the gene is repressed or when it is transcribed. Possible genes for our initial attempts include PHO5, CUP1, HIS4, genes of the GAL locus, and genes under control of the mating type locus.

Development and differentiation

While not at the level of addressing mechanisms of regulation, there are several genes under study in the laboratory that are involved in interesting features of development or differentiation. We have previously detailed the cloning of an exon of a putative Type IV collagen gene from Strongylocentrotus purpuratus. The gene is expressed at blastula and thereafter during development of the sea urchin. We have now examined the spatial distribution of expression of this collagen gene during embryogensis. At mesenchyme blastula, the 32 progeny of the micromeres which are piled up at the vegetal pole of the blastula actively express the gene. Other cells of the embryo either express the gene at a much lower level or not at all. Expression continues to occur exclusively in these cells as they enter the blastocoele to become primary mesenchyme and in the mature pluteus larva, where the cells have formed a synctium and elaborated the triradiate endoskeleton. This is the first primary mesenchyme specific gene described for the sea urchin. Of particular interest is the fact that micromeres, primary mesenchyme, and skeleton-forming cells can be isolated and can grow and differentiate in cell culture; this should facilitate study of the regulation of the collagen gene.

A second gene expressed during embryogenesis is that for ZP3, one of the zona pellucida proteins of the mouse. We have extensively characterized synthesis and processing of the zona proteins in years past and last year reported cloning of a cDNA for ZP3 from an expression library of murine ovarian RNA. ZP3 mRNA is 1.6 kb in length and is expressed uniquely in ovarian tissue. In situ hybridization has now demonstrated that expression of the gene occurs

only in oocytes, not granulosa cells. Expression does not occur in small, resting oocytes, is maximal in actively growing, medium sized oocytes, and appears to be largely repressed in fully grown oocytes; transcription thus closely parallels rates of zona protein synthesis determined by us previously.

We have localized the ZP3 gene to murine chromosome 6 using somatic cell hybrids. The gene is not amplified during oogenesis but does appear to be hypomethylated at CpG sequences in ovary as opposed to somatic tissues. We have screened several libraries in an attempt to obtain a full length cDNA clone (the current clones span about 80% of the message) without success. We have, however, recently isolated a genomic clone which appears to contain the ZP3 gene and several thousand base pairs of 5'-flanking sequences; this should allow investigations of the mechanism of control of transcription of this gene of importance in oogenesis.

Another set of genes involved in development are associated with a repetitive DNA element in Dictyostelium discoideum. The sequence ApApC is repeated tandemly up to 17 times at a number of locations in the genome of this simple eukaryote. Interestingly, the sequence is always on the coding strand; in some cases it is transcribed and in at least one case it is not. Genes associated with the repeat sequence are expressed at much higher levels during differentiation than in vegetative cells, although their regulation is not precisely coordinate. We have isolated several cDNA clones containng the repeat element and sequenced some of these. The repeat sequence in two of the clones is located in the 3' untranslated region of the gene, in contrast to the propositus gene in which the repeat is 5' to the message. One of the clones has reasonable homology over part of its sequence to the ras oncogene - the area of homology is in what is thought to be a GTP-binding protein domain.

Cyclic AMP plays an important role in development of Dictyostelium. We have previously reported that cAMP appears to act in two distinct fashions to regulate gene expression during differentiation. Some genes are regulated by intracellular cAMP, presumably through the protein kinase mechanism thought to function in cells of higher organisms. Other genes, however, appear to be regulated by a different second messenger with extracellular cAMP, interacting with a cAMP receptor on the cell surface, acting as a paracrine hormone.

We have studied possible mechanisms for this second type of regulation and have recently found evidence that regulation may occur using a pathway mediated by calcium, as suggested for other cAMP-independent hormonal responses in higher eukaryotes. First, we confirmed, using cAMP analogues, that "leaking" of extracellular cAMP into cells incubated with high exogenous concentrations of the nucleotide was unlikely to explain effects on gene regulation.. Thus, 2'-deoxy-cAMP, a cell surface receptor specific analogue, induced late gene expression while 8-Br-cAMP, which has a low affinity for the surface receptor, had a correspondingly low ability to induce these genes. Using permeabilized cells, we show that cells incubated under conditions which preclude accumulation of extracellular cAMP do not differentiate properly. In contrast, such cells aggregate normally when inosine-tris-phosphate is added to the medium; IP_3 can thus bypass the requirement for high extracellular concentrations of cAMP in differentiation. We suggest that stimulation of phospholipase C, production of PIP_2 and diacyl glycerol and calcium release may be causal in the paracrine action of cAMP during Dictyostelium development.

We are also attempting to isolate the gene for the cAMP receptor using antibodies to the receptor and cDNA libraries constructed from mRNA isolated from differentiating cells at the time of maximal receptor synthesis. Two independent cDNA clones have been isolated; both code for peptides which can affinity purify antibody specific for the cAMP receptor. Confirmation that these clones indeed are those derived from cAMP receptor mRNA awaits protein and DNA sequence information.

Cyclic AMP also appears to be involved in differentiation of certain higher cells. A system studied in this laboratory is MDCK (dog kidney) cells transformed with Harvey murine sarcoma virus. The parental cells respond to glucagon; this responsiveness is abolished after transformation. A variety of agents lead to reestablishment of the glucagon-sensitive state, among them prostaglandin E2, which strongly stimulates adenylate cyclase in MDCK cells. A role for cAMP was also suggested by observations previously reported that 8-Br-cAMP was an inducer of glucagon responsiveness. A rapid, transient increase in activity of one or both of the cAMP-dependent protein kinases occurs after addition of the prostaglandin to MDCK cells. Epidermal growth factor (EGF) inhibits prostaglandin or 8-Br-cAMP induction of glucagon responsiveness; surprisingly it does not alter cellular cAMP nor does it alter the increase in adenylate cyclase activity resulting from exposure to prostaglandin. As noted last year, this suggests that EGF inhibition of the differentiation process must occur distal to the site of action of cAMP. The complexity of interactions is further indicated by recent observations which show that EGF binding to MDCK cell receptors is decreased by 80% when cells are cultured in the presence of prostaglandin E2.

Lipid metabolism and transport

A model system for differentiation of adipocytes is provided by 3T3-L1 cells. We have previously studied synthesis and secretion of lipoprotein lipase (LPL) activity in these cells. Now, using an antibody to bovine LPL which cross reacts with the murine enzyme, we have studied synthesis of LPL protein in these cells and in mice with combined lipase deficiency. LPL was present in 3T3-L1 fibroblasts only after confluence. The amount of enzyme increased many fold as the cells differentiated into adipocytes; parallel increases in LPL activity and secretion were noted. Half of the lipase released was inactive. Heparin release studies how that about half of the lipase associated with cells is on the cell surface.

By analogy with glycerophosphate dehydrogenase, another enzyme needed for accumulation of fat, we suspect that the increase in LPL reflects transcriptional activation of the LPL gene. Tunicamycin blocked completely release of lipase to the medium and blocked cellular lipase activity nearly completely; the drug also led to production of a smaller LPL (Mr 48,000 vs 55,000 normally) due to arrest of N-glycosylation of proteins. Post-translational modification of LPL thus appears to be necessary for catalytic activity and secretion of the enzyme; the nature of the carbohydrates necessary for this modification(s) is unknown.

Combined lipase deficiency (cld/cld) is a recessive mutation in mice which leads to massive hyperlipemia and death within three days if the pups are allowed to suckle. Normal mice have high levels of hepatic lipase and LPL in liver and of the hepatic enzyme in plasma. In contrast, cld/cld mice lacked hepatic lipase in liver and plasma, lacked LPL in plasma but had 40% of normal values of LPL in liver. We note the possibility that the mutation may relate to glycosylation necessary for activity of hepatic and secretion of lipoprotein lipases. LPL is very low in extrahepatic tissues of cld/cld mice; apparently regulation of synthesis or modification of LPL differs for liver of neonatal mice vs. other tissues.

We have carried out immunocytochemical studies of LPL in brown adipose tissue of cld/cld mice. This tissue has very low levels of LPL activity but four times more immunoreactive LPL protein than in normal mice. Cultured brown adipose tissue cells from mutant animals were highly heterogeneous in shape and structure. Current studies of LPL localization in such cells are only at the light microscopic level and confirm the near absence of LPL protein in normal and presence of LPL protein in mutant adipocytes. Such studies will be extended to electron microscopic resolution to localize the protein in organelles. That such studies are feasible has been shown by similar investigations of LPL localization in mouse heart using gold- or ferritin-labeled second antibodies. LPL is found in capillaries along the luminal surface, although capillary endothelium is not believed to synthesize the enzyme. Myocytes are labeled with anti-LPL in organelles involved in synthesis, processing and secretion of glycoproteins, suggesting that these cardiac cells are a source of LPL in heart.

This laboratory has previously presented a model for fatty acid transport which suggest lateral movement in an interfacial continuum of external leaflets of intracellular and plasma membranes. It was proposed that fatty acids produced by lipolysis enter this continuum and form rivulets that flow around protein islets and between boundary lipids. Recently, we have used freeze fracture electron microscopy to provide evidence that fatty acids, produced by isoproterenol stimulated lipolysis, can form protein paricle free areas in the external leaflet of membranes in mouse adipose tissue. At high pH values (9.0), myelin figures were formed in intracellular channels, extracellular spaces and the capillary lumen. The E fracture face of plasma membranes of adipocytes and endothelial cells and of intracellular membranes of fat cells contained areas free of intramembranous particles. This suggests that the particle free areas are composed of partially ionized fatty acids located in the external leaflets of plasma and intracellular membranes of adipocytes and endothelial cells.

We have continued studies on movement of lipids in model membranes formed with fatty acids in a modified Langmuir trough. Recent results indicate that both protonated and partially ionized long chain fatty acids can flow in a lipid monolayer, equivalent to a single leaflet of a lipid bilayer existing in cells in vivo. Spreading pressure of long chain fatty acids at physiological pH values is equivalent to that of phospholipids; thus, we suggest that fatty acids could enter into and be transported through an interfacial continuum of cell membranes. Further, through its effects on spreading pressure, pH could regulate the entry into and the spread within the continuum of fatty acids.

Protein chemistry

Two areas of research in LCDB, in addition to the barnase project reviewed above, concern themselves with the biochemistry of physiologically important proteins, dihydrofolate reductase (DHFR) and apolipoprotein B (apoB). DHFR is the target enzyme for methotrexate, a drug initially used for cancer chemotherapy but now additionally employed for arthritis and some autoimmune disorders. Hepatic toxicity is a major complication of therapy with methotrexate and related drugs. We have studied the properties of DHFR from a variety of species for many years. Currently, we are attempting to detail the chemistry of human liver DHFR in order to relate its properties to those of better studied enzymes and, perhaps, optimize therapeutic effects and minimize toxic features of inhibitors of the enzyme.

We obtained autopsy specimens of human liver and were unable to detect DHFR activity in crude homogenates. In contrast, samples obtained during surgery and frozen immediately had low but detectable activity of DHFR in homogenates prepared in the presence of proteolytic inhibitors. The level of activity was approximately 1% of that present in bovine liver. Partial purification of human DHFR using methotrexate-Sepharose allowed determination of its isoelectric point as 6.8-7.0 and apparent molecular weight as ca. 22,000, values similar to those obtained for other vertebrate species.

Apolipoprotein B is of particular importance in metabolism of lipoproteins; it serves as the major structural protein of very low density lipoproteins (VLDL) and low density lipoproteins (LDL), the major transport vehicles for cholesterol and triacylglycerols. ApoB is essential for synthesis and release of VLDL and funtions in VLDL and LDL catabolism. ApoB is synthesized by liver and small intestine; three forms of apoB, differing in apparent molecular weight, are synthesized by liver (B-100, B-95, and B-48). We have developed a method for study of the relative rates of synthesis of these isoforms and response of same to diet and other physiological variations. Infusion of a nonionic detergent, Triton WR-1339, blocks clearance of pulse-labeled apoB from blood, allowing accurate measurements of synthetic rates in radioisotope pulse studies. Relative production rates of the isoforms were measured following Triton infusion, surgery or sham surgery, fasting, or intraduodenal infusion of glucose. Data show that the ratio of B-100/B-95 is decreased by physiological stress while the ratio of B-48/(B-100 + B-95) is decreased by fasting and increased by feeding.

Applied biology

The basic experiments reviewed above all have as an aspiration the possibility that their results may lead to some finding of benefit for mankind in health maintenance. Two areas of work in LCDB are closer to applied biology than the fundamental studies described previously.

Noted before were the studies of ZP3, one of the proteins which form the extracellular glycocalyx of the murine egg. Evidence from other laboratories has suggested that ZP3 is the sperm receptor of the murine egg. We have previously shown that passive immunization of mice with anti-ZP3 monoclonal antibodies led to prevention of pregnancy. The contraceptive effect was reversible - after shedding of oocytes coated with antibody at the time of immunization and maturation of juvenile oocytes which lacked zona proteins at the time of immunization, pregnancy was again possible.

We have now begun attempts to develop an active contraceptive vaccine. Using information derived from cloning and sequencing the ZP3 gene, we have produced a β-galactosidase-ZP3 fusion protein and isolated same. We have also synthesized two peptides containing likely epitopes of ZP3 and coupled them to carrier proteins. We are in the process of testing these three reagents as possible contraceptive vaccines. While the current studies are in mouse, cross reactivity of the ZP3 cDNA clone with a variety of vertebrate species suggests that, if successful, such a strategy could rapidly be extended to domestic animals and perhaps eventually to humans.

The Biotechnology Unit of LCDB is engaged in large scale fermentation, tissue processing, and protein purification in support of various groups within NIH. A total of 115 large scale preparations were carried out in the past year. A number of eukaryotic and prokaryotic microorganisms were grown and processed in volumes varying from 10 to 1200 liters. Additionally, several large scale purifications were done by the staff of the Biotechnology Unit and mammalian tissue culture cells were provided to several research groups in volumes up to 50 liters. While this service facility, unique on the NIH campus, provides materials and expertise for a variety of scientists, this group also performs research and development functions in several areas. This year work has continued in computer controlled fermentation of bacterial and animal cells and on optimization of production of toxins important for generation of new, safer vaccines for infectious diseases.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 15004-11 LCDB
formerly
Z01 AM 15004-10 LCDB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regulation of Hormone Responsiveness During Cellular Differentiation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Michael C. Lin	Research Chemist	LCDB, NIDDK
Others:	Yvonne Wu	Senior Staff Fellow	LCDB, NIDDK

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Cellular and Developmental Biology

SECTION
Membrane Regulation Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	1.0	0.8

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Glucagon responsiveness was selectively lost in a dog kidney cell line, MDCK cells, after transformation by Harvey murine sarcoma virus and this loss can be restored to the transformed cells by culturing the cells in the presence of prostaglandin E_2. The induction by PGE_2 seems to be mediated by cyclic AMP. We are currently examining the role of cyclic AMP-dependent protein kinase in the induction process. In order to define the nature of this cyclic AMP-dependent process, we have studied several differentiation inhibitors which inhibit the induction of glucagon responsiveness by PGE_2. Epidermal growth factor inhibits the induction by PGE_2 in a concentration dependent manner, but does not have detectable effect on the ability of PGE_2 to activate cyclic AMP production. We have also found that EGF receptors become desensitized during the induction. It has been shown that EGF receptors can be phosphorylated not only by a EGF-induced process but also by a cyclic AMP-dependent pathway. We have now identified the EGF receptors in the transformed MDCK cells on SDS-PAGE by ^{35}S-methionine labeling followed by immunoprecipitation. We are currently examining the phosphorylation of EGF receptors under the induction condition and when the induction of differentiation is inhibited.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 15005-11 LCDB
formerly
Z01 AM 15005-10 LCDB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regulation of Adipocyte Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.5	5.0	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Adipocytes from rat epididymal rat pads contain a complex of receptors that either stimulate (R_s) or inhibit (R_i) adenylate cyclase and, within the plasma membrane, the actions of these receptors are regulated by their respective GTP-binding proteins, N_s and N_i. Previously, we demonstrated that ligands for R_s and R_i receptors regulate cellular responses, such as the activity of the glucose transporter, in a manner independent of changes in cellular cAMP concentrations. Treatment of fat cells with cholera toxin to modify N_s, and treatment with pertussis toxin to modify N_i, reveals that in regulating their adenylate cyclase-independent actions on the glucose transporter, the R_s and R_i receptors act via the same GTP-binding proteins used to regulate adenylate cyclase activity.

In order to continue our examination of the mechanism whereby insulin inhibits lipolysis in the face of elevated cellular cAMP-dependent protein kinase activity, we have purified the hormone sensitive lipase from fat cells. By drawing on experience gained from purification of the adrenal cholesterol esterase, thought to be identical to the fat cell lipase, a rapid purification scheme was developed, based both on selective management of the detergent environment and on developing procedures for early removal of cytoskeletal proteins in cell extracts.

Previously, we showed that adenylate cyclase activity in purified adipocyte membranes is stimulated by the calcium/phospholipid-dependent enzyme, protein kinase C. Since the effect of the kinase on adenylate cyclase occurs under conditions highly unfavorable for phosphorylation, we conclude that the kinase associates with and thus activates cyclase.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 15100-16 LCDB
formerly
Z01 AM 15100-15 LCDB

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Protein-Nucleic Acid Interactions: Chromatin Structure and Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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	P. FitzGerald	Staff Fellow	LCDB	NIDDK
	R. Morse	NRSA Fellow	LCDB	NIDDK
	D. Pederson	Staff Fellow	LCDB	NIDDK
	T-C. Wu	Chemist	LCDB	NIDDK
	J.M. Brubaker	Biological Lab. Tech.	LCDB	NIDDK

LAB/BRANCH

Laboratory of Cellular and Developmental Biology

SECTION

Developmental Biochemistry Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
10.0	8.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Methodology has been developed to allow purification of yeast plasmid chromatin to apparent biochemical homogeniety. Several types of experiments have shown that the purified material is representative of >90% of the total plasmid population and that the material is transcriptionally active. Minichromosomes containing TRP1ARS1 yeast sequences contain seven nucleosomes (detected by both electron microscopy and determination of linking number), in agreement with earlier nuclease mapping data. Histones are the major protein constituents of the minichromosomes. Preliminary results indicate that purification of the minichromosome by a method using protein-nucleic acid affinity is feasible; this procedure is simpler and quicker than the purification using conventional biochemical techniques.

Experiments aimed at understanding chromatin structure of regulated genes continue. The chromosomal organization of the CUP1 gene in yeast coding for a metal-inducible metallothionein-like protein has been determined for both basal and induced states. We have previously described changes in the organization of sea urchin histone genes during developmentally regulated transcription. One of the hypersensitive 5'-flanking regions of these genes selectively binds to a 55000 dalton protein which is present only when the genes are active.

A Type IV collagen gene exon from Strongylocentrotus purpuratus has been cloned and sequenced. This gene is developmentally regulated, turning on at blastula. We have determined the spatial distribution of expression of the gene; synthesis of the collagen mRNA is confined largely, if not exclusively, to primary mesenchyme cells throughout their differentiation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 15102-26 LCDB
formerly
Z01 AM 15102-25 LCDB

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Study of a Ribonuclease and its Inhibitor from Bacillus amyloliquefaciens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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Laboratory of Cellular and Developmental Biology

SECTION
Developmental Biochemistry Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	2	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Two proteins, barnase, the extracellular ribonuclease of Bacillus amyloliquefaciens, and barstar, its intracellular inhibitor, are used as a model system for the study of protein folding a protein-protein interactions. Barnase is one of an homologous group of ribonucleases occurring in both prokaryotes and eukaryotes.

Recombinant DNA techniques are being applied to the project with three major aims; 1) to facilitate production, 2) to examine the structural and control sequences of the genes and 3) to tailor specifically designed modifications in the sequences to test theories of protein folding.

The complete barnase gene is lethal when reassembled in either E. coli or B. subtilis. With directed mutation of the essential histidine-102 the gene is expressed in both organisms, but the mutant products are not correctly processed. A vector has been devised, based on the promoter and signal sequence of the Pho-A gene, which allows facile production of the muture (mutant) enzyme in high yield (16-20 mg/liter).

The barstar gene has also been cloned in E. coli, where it is expressed as a specific inhibitor of barnase.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 15200-26 LCDB
formerly
Z01 AM 15200-25 LCDB

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies on Folic Acid (Dihydrofolate Reductase) and Vitamin A

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: Bernard T. Kaufman Research Chemist LCDB, NIDDK

COOPERATING UNITS *(if any)*

Dr. John Bieri, Scientist Emeritus, NIDDK
Dr. Carmin Allegra, NCI

LAB/BRANCH

Laboratory of Cellular and Developmental Biology

SECTION

Nutritional Biochemistry Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	2	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standerd unreduced type. Do not exceed the space provided.)*

With the increasing use of methotrexate (MTX) to treat arthritis, liver toxicity, which limits use of this drug, is of increasing concern. Because dihydrofolate reductase (DHFR) is the specific site of inhibition of this folic acid antagonist, the function of DHFR in liver, particularly human liver, is an important research objective. We are continuing our studies to isolate and characterize hepatic DHFR from human liver samples as well as from other species. We have found differences in human liver DHFR activity depending upon the tissue source. Not surprisingly, DHFR activity could not be detected in preparations of human liver from autopsies. Human liver samples obtained during surgery, on the other hand, gave measurable levels of DHFR activity when homogenized with appropriate proteolytic enzyme inhibitors. The DHFR levels of the human liver samples tested to date are quite low compared to preparations of chicken, beef, and sheep liver, i.e. 1/100 the level of activity in beef. Human hepatic DHFR, partially purified by affinity chromatography on MTX-sepharose followed by isoelectric focusing, has a mol. wt. of 22,000 and an isoelectric point of 6.8-7.0.

Continuation of the studies to obtain more detailed information on carotenoid metabolism in normal humans: HPLC analysis of human plasma gave a peak of some 10-15% of total carotenoids different from any known standard compounds. Current studies suggest that the unknown compound may be hydroxy-alpha-carotene.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 15302-16 LCDB
formerly
Z01 AM 15302-15 LCDB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biochemical Studies of Hepatic and Intestinal Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Dr. H.G. Windmueller	Research Chemist	LCDB	NIDDK
Others:	Mr. A.E. Spaeth	Chemist	LCDB	NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Cellular and Developmental Biology

SECTION
Nutritional Biochemistry Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	0.4	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are continuing to use the rat as a model to study the biosynthesis and metabolism of plasma apolipoprotein B (apoB), the structural protein of very low density lipoproteins (VLDL). Evidence has been obtained that perfused rat livers produce three forms of apoB (B-100, B-95 and B-48), differing in their apparent molecular weights. Furthermore, the relative amount of the three forms produced appears to be regulated by diet and perhaps other physiological variables. This seems important because in other studies we have shown that all three forms of apoB are independently metabolized. Together, these findings point to a complex mechanism in the rat whereby the metabolic fate of hepatic VLDL and its lipid component is physiologically regulated by the form of apoB it contains.

We have developed a new and simpler method in vivo to measure the relative production rates of the individual forms of apoB. The method is based on the ability of Triton WR-1339, a nonionic detergent given I.V., to block the clearance of pulse-labeled apoB from the circulation. Findings with this method are as follows (all ratios refer to relative production rates): (1) Triton alone reduced B-100/B-95 from 3 to 2 without affecting B-48/(B-100 + B-95). (2) Surgery to implant cannulas in the jugular vein and duodenum, followed by restraint, reduced B-100/B-95 to 1.3 after 18 hours without affecting B-48/(B-100 + B-95). Sham surgery alone had a similar effect after the same time period. (3) Infusing glucose intraduodenally for 18 hours increased B-48/(B-100 + B-95) without affecting B-100/B-95. (4) Fasting for 42 hours decreased B-48/(B-100 + B-95) from 2.9 to 0.7 without affecting B-100/B-95. (5) Taken together these data indicate that B-100/B-95 is decreased by physiological stress while B-48/(B-100 + B-95) is regulated by dietary variables.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 15400-12 LCDB
formerly
Z01 AM 15400-11 LCDB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Hormones, Lipoprotein Lipase and Lipid Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnal below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Robert O. Scow	Chief, Endocrinology Section	LCDB, NIDDK
Other:	Sidney S. Chernick	Scientist Director	LCDB, NIDDK
	Mary M. Garrison	Physiologist	LCDB, NIDDK

COOPERATING UNITS *(if any)*
Dr. Thomas Olivecrona, Dept. of Physiol. Chem., Univ. of Umea, Sweden; Drs. W. Virgil Brown and Kazuhiro Oka, Division of Atherosclerosis and Metabolism, Mt. Sinai School of Medicine, New York, NY 10029

LAB/BRANCH
Laboratory of Cellular and Developmental Biology

SECTION
Endocrinology Section

INSTITUTE AND LOCATION
NIH, NIDDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.3	1.3	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Murine 3T3-L1 cells were used to study synthesis and secretion of lipoprotein lipase in adipocytes. Lipase protein was present in confluent, but not preconfluent, 3T3-L1 fibroblasts. The amount of enzyme in the cells increased many fold as cells differentiated into adipocytes. This increase was accompanied by parallel increases in lipase activity and secretion. The amount of [S-35] methionine incorporated into lipase by 3T3-L1 adipocytes increased for 2 h and then remained constant, indicating a half-life of 1 h for newly synthesized lipase. Lipoprotein lipase released from cells by heparin was maximally active and accounted for 40% of active lipase associated with the cells. Tunicamycin reduced total protein synthesis 25%, increased cellular lipase protein 75%, decreased cellular lipase activity 99%, and blocked completely release of lipase to the medium. Molecular weight of [S-35]-labeled lipase synthesized in the presence of tunicamycin was smaller on SDS-PAGE than that synthesized by control cells, 48,000 vs 55,000, due to arrest of n-glycosylation of protein in endoplasmic reticulum. The complement of carbohydrates needed for full activity and secretion of lipoprotein lipase requires study.

A preliminary finding that hepatic lipase activity was very low in liver and plasma of Combined Lipase Deficient (cld/cld) mice was confirmed by immunoinhibition studies. Lipoprotein lipase activity, which was very low in extrahepatic tissues, was unexpectedly high, 40% of normal, in liver of cld/cld mice, suggesting that genetic control of this lipase in liver differs from that in other tissues.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 15401-14 LCDB
formerly
Z01 AM 15401-13 LCDB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Transport of Lipids, Hormones and Enzymes in Tissues, Cells and Membranes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Robert O. Scow	Chief, Endocrinology Section	LCDB, NIDDK
Others:	E. Joan Blanchette-Mackie	Research Biologist	LCDB, NIDDK
	Sidney S. Chernick	Scientist Director	LCDB, NIDDK
	Lynne Amende	Senior Staff Fellow	LCDB, NIDDK
	Nancy K. Dwyer	Biologist	LCDB, NIDDK

COOPERATING UNITS *(if any)*
W. Virgil Brown, Professor, and Kazuhiro Oka, Assistant Professor, Division of Atherosclerosis and Metabolism, Mt. Sinai School of Medicine, New York, N.Y. 10029

LAB/BRANCH
Laboratory of Cellular and Developmental Biology

SECTION
Endocrinology Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.2	1.7	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our laboratory has developed a model for transporting fatty acids in tissues by lateral movement in an interfacial continuum of external leaflets of intracellular and plasma membranes. When oleic acid is added to aqueous solutions, it covers the aqueous surface with a monolayer and the excess forms lenses (oil droplets) above the monolayer. We found that the spreading pressure of oleic acid increased from 34 to 44 dynes/cm when pH of the medium was increased from 5.4 to 7.4., demonstrating that affinity of fatty acids for lipid monolayers increased with pH. We studied the effect of pH on movement of oleic acid in a monolayer covering the surface of aqueous medium in a trough with interconnected compartments. When pH of the medium in one compartment was increased above that in the other compartments, fatty acid molecules in lenses above that compartment entered and moved in the monolayer to the other compartments where they formed lenses above the monolayer.

We found, using freeze-fracture electron microscopy, areas free of protein particles in external leaflets of intracellular and plasma membranes in adipose tissue processed at pH 9.0. The surfaces of these areas sometimes formed multiple folds that abutted on myelin figures. We concluded that these areas were formed by fatty acids that entered leaflets from lipolyzed lipid droplets in adipocytes and spread throughout a continuum of leaflets, and when they overcrowded the continuum, they produced foldings and lamellar extension of the continuum in the form of myelin figures. These findings support our model for transport of fatty acids in tissues.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 15404-02 LCDB
formerly
Z01 AM 15404-01 LCDB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Ultrastructural Immunolocalization of Enzymes in Cells and Tissues

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	E. Joan Blanchette-Mackie	Research Biologist	LCDB, NIDDK
Others:	Sidney S. Chernick	Scientist Director	LCDB, NIDDK
	Robert O. Scow	Chief, Endocrinology Section	LCDB, NIDDK
	Nancy K. Dwyer	Biologist	LCDB, NIDDK
	Lynne Amende	Senior Staff Fellow	LCDB, NIDDK

COOPERATING UNITS *(if any)*
Drs. W. Virgil Brown and Kazuhiro Oka, Division of Atherosclerosis and Metabolism, Mt. Sinai School of Medicine, New York, NY 10029

LAB/BRANCH
Laboratory of Cellular and Developmental Biology

SECTION
Endocrinology Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.0	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Lipoprotein lipase, a glycoprotein, acts on chylomicrons at the luminal surface of capillaries. Tissue cells other than endothelium are believed to synthesize the enzyme. Distribution of lipoprotein lipase in heart of fed and fasted mice was studied with an indirect immunocytochemical method using chicken antiserum to bovine milk lipoprotein lipase, which cross-reacts with mouse lipoprotein lipase, and gold- or ferritin-labeled antibodies. This approach demonstrates protein which could be either an active or an inactive form of the enzyme. Lipoprotein lipase was found in capillaries and cardiac myocytes. The gold labeling of the capillary surface located lipoprotein lipase at sites of activity in capillaries. The gold labeling of cardiac myocytes in organelles involved in the synthesis, of glycoproteins indicates that cardiac myocytes are a source of lipoprotein lipase. Fasting increased the amount of lipoprotein lipase, demonstrated immunocytochemically, in myocytes, extracellular space and capillaries.

Brown adipose tissue of mice with Combined Lipase Deficiency (cld/cld) have very low levels of lipoprotein lipase activity. Yet 4 X normal amounts of lipoprotein lipase protein. Cultured cells from brown adipose tissue of cld/cld mice were shown by immunofluorescence to contain intracellular lipoprotein lipase whereas cells from normal littermates contained none. These findings suggest that cell cultures of brown adipose tissue can be used to study synthesis and secretion of lipoprotein lipase.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 15405-04 LCDB
formerly
Z01 AM 15405-03 LCDB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Aggregation of Human Platelets Induced by Decompression: Mechanism and Prevention

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: Makio Murayama Research Chemist LCDB, NIDDK

COOPERATING UNITS *(if any)*
K.K. Kumaroo, Biochemist, U.S. Naval Research Institute, Bethesda, MD

LAB/BRANCH
Laboratory of Cellular and Developmental Biology

SECTION
Endocrinology Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We reported that human platelets aggregate when decompressed by reduced barometric pressure to 253 torr (Thrombosis Res. 33: 477-485) and that this aggregation was inhibited by menthol and methone at 10 mM and by thymol, an aromatic derivative of menthol, at 5 μM (Thrombosis Res. 42: 511-516, 1986). A theory of spontaneous aggregation of human platelets by decompression was derived from our observation that the molecular volume of activation of fibrin polymerization is a large positive number. Therefrom, it was deduced that the rate of fibrin polymerization could be accelerated by decompression. This was experimentally tested and confirmed by producing spontaneous aggregation ofplatelets decompressed by various means. The corollary to the theory is that platelets generally do not aggregate when compressed. Recently, we confirmed our previous observation that the rate of fibrin polymerization was essentially doubled at 1/2 atm compared to the control at 1 atm. Furthermore, it was found that fibrin polymer "melts" under high hydraulic pressure (10,000 psi). Fibrin polymerization appears, therefore, to be a reversible process as predicted by theory (Murayama and Nakada). The pressure effects on fibrin and platelet aggregation demonstrate that the underlying mechanisms involve electric constriction and that electrically charged residues are involved. The design of future experiments is directed towards solving the electrostriction problem(s) in fibrin polymerization as related to platelet aggregation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 15500-26 LCDB
formerly
Z01 AM 15500-25 LCDB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Large Scale Processing of Biological Material

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Joseph Shiloach	Research Chemist	LCDB, NIDDK
Others:	Jeanne E. Kaufman	Biol. Lab. Tech.	LCDB, NIDDK
	Nahum Andorn	Visiting Fellow	LCDB, NIDDK
	Ilse Blumentals	Guest Worker	LCDB, NIDDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Cellular and Developmental Biology

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Pilot Plant is involved in large scale growth of microorganisms and mammalian cells, and in large scale isolation and purification of biologically active components from these sources and from other sources such as mammalian or plant tissues. We assist NIH investigators in the scaling up of processes by conducting research and development work for the appropriate approach suitable of the large scale processing.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 15502-05 LCDB

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Relationship Between Differentiation and Transformation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Suzanne K. Beckner Senior Staff Fellow LCDB, NIDDK

COOPERATING UNITS *(if any)*

Thomas Y. Shih, Research Scientist, LMO/C
William L. Farrar, Senior Staff Fellow, LIR/C

LAB/BRANCH

Laboratory of Cellular and Developmental Biology

SECTION

Section on Membrane Regulation

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 15503-05 LCDB formerly Z01 AM 15503-04 LCDB

PERIOD COVERED: October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*: Regulation of Developmental Gene Expression

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Alan R. Kimmel	Senior Staff Fellow	LCDB, NIDDK
Other:	Charles Saxe	Staff Fellow	LCDB, NIDDK
	Stephen Saxe	Staff Fellow	LCDB, NIDDK
	Michael Eisen	FAES Fellow	LCDB, NIDDK

COOPERATING UNITS *(if any)*: None

LAB/BRANCH: Laboratory of Cellular and Developmental Biology

SECTION: Developmental Biochemistry Section

INSTITUTE AND LOCATION: NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.2	3.2	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Cellular proliferation and differentiation are responses elicited by interaction of extracellular molecules with the cell surfaces of eukaryotic cells. In Dictyostelium extracellular cAMP acts similarly to paracrine hormones in mammalian cells by interacting with a specific cell surface receptor to stimulate the synthesis of intracellular second messages. Our results indicate that there are at least two independent mechanisms involved in the developmental regulation of gene expression by cAMP in Dictyostelium. Conditions which allow intracellular synthesis of cAMP promote the normal regulation of a gene known to be repressed in conjunction with cAMP signalling. In contrast, expression of genes which exhibit maximal activity after aggregate formation depends upon accumulation of extracellular cAMP and not intracellular cAMP signalling. We have additionally shown that extracellular cAMP can be at least partially bypassed in permeabilized cells exposed to inositol tris phosphate (IP_3), a mobilizer of intracellular calcium ion; we suggest that IP_3 acts as a second messenger in Dictyostelium to mediate certain developmental processes. Finally, we have provisionally isolated a gene for the cAMP cell surface receptor and have also cloned a gene which has homology with the α subunit of the GTP-binding, N-regulatory protein and with the ras family of proto-oncogenes. Studies now focus on their expression and function in order to understand the molecular mechanisms of signal transduction.

PHS 6040 (Rev 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 15505-03 LCDB

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Role of GTP-Regulatory Proteins in Regulation of Membrane Processes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Martin Rodbell Section Chief LCDB, NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Cellular and Developmental Biology

SECTION

Section on Membrane Regulation

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 15506-03 LCDB
formerly
Z01 AM 15506-02 LCDB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Control of Gene Expression in Early Mammalian Development

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Jurrien Dean	Senior Investigator	LCBD, NIDDK
Others:	Maurice Ringuette	Visiting Fellow	LCDB, NIDDK
	Steven Chamow	Staff Fellow	LCDB, NIDDK
	Margaret Chamberlin	FAES Graduate Student	LCDB, NIDDK
	Caroline Philpott	Howard Hughes Scholar	LCDB, NIDDK

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Cellular and Developmental Biology

SECTION
Developmental Biochemistry Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	5.0	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Although the one-cell zygote has a large complement of mRNAs synthesized during oogenesis, the extent to which these maternal genes modulate early development is unknown. We have focused our investigations on understanding the molecular details of oocyte-specific genes and have chosen as our model system the genes that code for three sulfated glycoproteins of the mouse zona pellucida (ZP-1, ZP-2, and ZP-3). We have isolated cDNAs coding for ZP-3, the mouse sperm receptor, from an ovarian cDNA library cloned in the λgt-11 expression vector. The identity of these clones was confirmed by a comparison of their nucleic acid sequence with the amino acid sequence of the ZP-3 protein. We have shown that the ZP-3 message is expressed as a 1.6 kb poly-adenlyated mRNA which is found uniquely in ovarian tissue. Furthermore, from Northern analysis and in situ hybridizations, it appears that ZP-3 is expressed only in oocytes and not in surrounding granulosa cells. Based on our in situ data, the gene does not appear to be transcribed in resting oocytes (17 μm) but rapidly becomes a very abundant message in growing oocytes (50 μm) before being turned off in fully grown oocytes (70 μm). Thus, the transcription of the gene appears to closely parallel our previous observations on zona protein biosynthesis during oogenesis. Somatic cell hybrids were used to localize the ZP-3 locus to mouse chromosome 6 where it appears to exist as a single copy gene. Although the structure of the gene is the same in germ-line and somatic tissues, the ZP-3 gene is hypomethylated in ovarian tissue (where it is expressed) compared to somatic tissue. We have recently isolated a genomic clone contain-

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 15507-08 LCDB
(formerly
Z01 AM 27009-07 LPB)

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Macromolecular Structure

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Alasdair C. Steven, Visiting Scientist - Section Chief — LCDB, NIDDK

Adelia C. Bauer, Physiologist — LCDB, NIDDK
Margaret E. Bisher, Microbiologist — LCDB, NIDDK
Colin D. Ockleford, Visiting Scientist — LCDB, NIDDK
David A.D. Parry, Guest Researcher — LCDB, NIDDK

COOPERATING UNITS *(if any)* B. Trus,DCRT; M. Unser,BEIB; P. Ross, LMB,NIDDK; J. Cowell, FDA; J. Brown & W. Newcomb, U.Va.; L. Black, U. Md.; J. Maizel & P. Steinert, NCI; R. Podolsky, NIAMS; Co. Steer, LMB,NIDDK; F. Studier, Brookhaven Nat'l Lab., N.Y. FOREIGN: A. McDowall & J. Dubochet, EMBL, Heidelberg, R.R.G.

LAB/BRANCH
Laboratory of Cellular and Developmental Biology

SECTION
Section on Structural Biology

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.11	2.11	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The structural and compositional heterogeneity of coated vesicles purified from rat liver has been investigated by compiling mass distributions of LCV and of particles derived from them both by extraction with the non-ionic detergent Triton X-100 and by removal of their clathrin coats by manipulation of pH and ionic strength. The masses of individual particles were determined by computer image processing of dark-field scanning transmission electron micrographs of unstained frozen-dried preparations; these data were compiled into statistical distributions and the resulting data were then correlated with biochemical determinations of the global contents of protein, phospholipid, and cholesterol in the respective preparations. We found that ~ 30% of "LCV" do not, in fact, contain membrane vesicles (> 70% in the case of brain coated vesicles), but have proteinaceous cores. The remaining 70% contain vesicles seemingly of two kinds differentiated according to the relative amounts of material contained within their lumens. Vesicle-containing LCV are markedly heterogeneous, with masses varying over a four-fold range (~50 to 200 megadaltons-MDa) and a two-fold diameter range (~80 to 160nm). The average mass of such particles is 80 MDa, of which 46 MDa is contributed by clathrin and other coat proteins, 10-12 MDa by phospholipid and cholesterol, and 20-22 MDa by vesicle proteins. As estimated by SDS-PAGE the latter constitute an extremely diverse collection of proteins that are present in very small amounts, except for a relatively prominent doublet of 46kDa. In addition, structural analyses based on various electron microscopical methods reinforced by digital image processing have been performed on a number of other biological systems, including keratin intermediate filaments, the myofilament lattice of vertebrate skeletal muscle, the fimbriae of Bordetella pertussis, and the tail-fibers of bacteriophage T7.

GPO 914-918

ANNUAL REPORT OF THE LABORATORY OF BIOCHEMISTRY AND METABOLISM

NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

The Laboratory conducts research in such apparently disparate areas as differentiation, morphogeneisis, endocytosis, endocrinology, membrane transport, detoxication and protein behavior. Such seemingly distant relations are emphasized by the very different methods that are being applied to solve the enumerated problems. Resolution is being attempted by approaches that stem from enzymology, carbohydrate chemistry, cell biology and molecular biology. Although such diversity may seem chaotic, there is actually a common element to each of the subjects summarized here that is appropriate to the laboratories' designation: biochemical and metabolic approaches are being brought to bear on major problems encompass by the Institute's charge. It is the close proximity of experienced investigators from diverse scientific disciplines, discussing their distinct approaches to a given problem with each other, that provide synergistic effects for the resolution of problems under investigation.

A. Growth, Differentiation and Morphogenesis

Several groups are active in this broadly designated area by exploring different aspects of development of mammary tissue and its distinct protein products, as well as an investigation of the origin and development of the bud scar of yeast.

1. Hormone-dependent Development of Mammary Gland

The molecular and cytological events involved in the development of the mammary gland are being explored. Although estrogen has long been recognized as an essential for mammary cell proliferation, it was assumed that the

steroid plays no positive role in milk protein gene expression until recently. It has now been demonstrated that estrogen depleted *in vivo* renders mouse and rat mammary cells virtually incapable of such gene expression *in vitro* in response to lactogenic hormones, and that this defect can be corrected by administration of estrogen *in vivo*. The role of estrogen in mammary differentiation has been further analyzed using the rabbit system. Again, the cells isolated from estrogen-depleted animals have virtually no initial ability to synthesize casein or α-lactalbumin in response to insulin, cortisol and prolactin, although they retain full general responsiveness to prolactin. However, after extended culture in the absence of exogenous estrogen, the cells recover fully their ability of synthesizing the milk proteins. By contrast, α-lactalbumin is not synthesized *in vivo* in the presence of elevated prolactin levels unless estrogen is administered. The results are being interpreted as follows:
1) A factor(s) which inhibits induction of milk proteins by lactogenic hormones is present in the mammary gland when estrogen is deficient; 2) the putative factor initially present in the isolated, estrogen-deficient mammary tissue is dissipated after extended culture even in the absence of exogenous estrogen; 3) the inhibitory factor(s) remains in the mammary tissue *in vivo* until estrogen is administered.

Comparison of insulin (I) and IGF-I on mouse mammary cells, in the presence of cortisol and prolactin, shows: 1) Both I and IGF-I, at physiological levels, can induce the glucose transport system up to the level in the two-day lactating animal. Together, they can induce it to the ten-day lactating level, i.e., the effects are additive; 2) the factors can induce the same maximum level of alpha-lactalbumin activity, but the specific biological activity of I is 15 times greater than that of IGF-I; 3) I, but not IGF-I, can markedly enhance casein gene expression above the mid-pregnant level.

2. Tissue Specific and Hormonal Regulated Gene Expression.

Here the approach is mainly from the discipline of molecular biology whereby the milk protein system is being used as a model for defining the cis-regulatory elements and trans-acting factors that determine the tissue specificity and hormone induced expression. Additionally, a study is under way of the mechanism of activation and repression imposed on the major immediate early gene of the human cytomegalovirus (HCMV) in different host cells.

The expression of milk protein genes in the lactating mammary gland is controlled by steroid and peptide hormones. The thrust of work is toward an understanding of the cis-element(s) and trans-acting factors that determine tissue specificity and hormone inducibility of the gene for the whey acidic protein (WAP). Studies on the interaction of nuclear proteins from mammary epithelial cells with the WAP gene promoter region revealed that the region between -10 and -200 is recognized specifically by at least four different proteins. In order to elucidate the functions of these potential trans-acting factors, an *in vivo* assay had to be developed. In collaboration with Groner's laboratory (Bern, Switzerland), transgenomic mice were established that carried the Harvey ras gene under the control of the WAP promoter. The fusion gene was expressed only in the lactating mammary gland, indicating that the promoter fragment contained the necessary regulatory elements. The female mice developed mammary tumors and can be used to study mammary tumorigenesis. These studies represent a novel demonstration of a specific sequence of nuclear proteins binding to a mammary specific promoter, and of tissue specific expression of this promoter in transgenomic mice.

Experiments have been begun to define the *in vivo* and *in vitro* activator and repressor elements that govern the expression of the first gene in the life cycle of human cytomegalovirus, a known pathogen. In collaboration with

the laboratory of Fleckenstein (Erlangen, FRG), sites of protein-DNA interaction between an enhancer and potential repressor are being defined.

3. Polysaccharides in Morphogenesis

The ongoing research attempts to provide an understanding of the molecular mechanism of morphogenesis. The topics currently under study are the formation of the primary septum of yeast, i.e., the bud scar, and the biosynthesis of a glucan, the latter a major structural component of the cell wall of yeast and other fungi.

Work on the cloning of the chitin synthetase gene of Saccharomyces cerevisiae, in collaboration with the laboratory of P.W. Robbins (M.I.T.), has continued. It has been confirmed that the cloned gene codes for the synthetase protein. It has also been found that strains harboring a disrupted chitin synthetase gene are viable and have a normal chitin content. This result suggested the presence of another chitin synthetase, which was subsequently found and characterized.

Studies on the dissociation of fungal ß(1-3)glucan synthetase into two fractions, one of which appears to interact with guanosine nucleotides, have been pursued further. The interaction with GTP-γ-S has been demonstrated in different ways. The results have been extended to S. cerevisiae, whose soluble component is now under purification.

B. Proteins and Enzymes

At the center of it all are the protein catalysts. In this laboratory, two groups are specifically and directly oriented toward protein chemistry and enzymology.

1. Thermodynamic and Kinetic Studies of Protein Structure and Enzymic Mechanisms.

Work is directed toward the relationship of protein sequence to conformation and enzyme activity. Since the digestive enzyme, pepsin, is

synthesized as an inactive precursor, pepsinogen, there is interest in the molecular basis of the proteolytic activity of the enzyme: how activity is suppressed in the zymogen and is induced during activation.

On exposure to acid pH, porcine pepsinogen undergoes self cleavage to produce pseudo-pepsin by the loss of the first 16 residues in its sequence. Returning to neutrality produces an irreversible conformational change in pseudopepsin to a new globular form, which seems to be identical to the rapidly formed transient intermediate previously detected during the folding of intact pepsinogen. Like pepsinogen, but unlike native pseudopepsin, this neutral pH form of the protein can be reversibly unfolded by urea or high pH. The mechanism of the folding reaction, the structure and stability of this neutral pH form of pseudo pepsin, and the role of the first 16 residues in the mechanism of folding of pepsinogen, are all under investigation.

2. Enzymatic Basis of Detoxication

One group is investigating the enzymes of detoxication, three dozen or so enzymes that are distributed ubiquitously among higher animals with the apparent function of detoxifying xenobiotics, i.e., foreign compounds. As a result of such efforts it is now becoming clear that these enzymes generally have two properties in common: 1) they function in a manmner that is designed to convert xenobiotics into readily excretable and pharmacologically inert compounds. 2) The enzymes themselves are characterized by a very broad substrate specificity with particular avidity for lipophilic ligands.

Under study at present are those enzymes that act on amines. Specifically, two isozymes of amine N-methyltransferase from rabbit liver have been obtained which catalyze with overlapping specificity, the transfer of methyl groups from S-adenosyl-L-methionine to a large number and variety of amines. Serving as methyl acceptors are primary, secondary and tertiary amines of very different carbon skeleton that include aliphatic, aromatic and

heterocyclic compounds. The presence of the enzyme in most animal organs, including brain, suggests the possibility of using pyridine derivatives as prodrugs that could pass such membranes as the blood brain barrier. Upon methylation within brain, the resultant pyridinium ion would remain within the cell to exert its pharmacological effect. It should be pointed out that toxic products are also possible: methylation of 4-phenylpyridine results in symptoms of Parkinsonism and methylation of 4,4'-aminobipyridyl yields the toxic paraquat. Both isoenzymes have now been prepared in homogenous forms and have been characterized.

C. Biochemistry, Function and Regulation of Membranes

Under the heading of membranes are a broad range of projects that range from nuclear membranes, through exocytosis and membrane transport, to the endocrinology of thyroid disease.

1. The Role of the Nuclear Envelope in Intracellular Protein Sorting.

An investigation is underway to develop an immunoaffinity isolation procedure for the preparation of the organelles involved in membrane and secretory glycoprotein assembly and to define the mechanisms involved in the entry of proteins through the nuclear membrane into the nuclear interior.

The mechanisms which allows localization of proteins to the nucleus and to the various organelles of the secretory pathway are under investigation. Using a temperature sensitive mutant of vesicular stomatitus virus it has been possible to synchronize intracellular transport of the single glycoprotein of the virus (G protein). The mutant G protein accumulates in the nuclear envelope and rough endoplasmic reticulum at 39°C. It has now been demonstrated that the nuclear envelope is not required for transport of G protein. Using an antiserum which recognizes only the cytoplasmic domain of this transmembrane protein, the organelles to which it is routed may be purified by immunoaffinity isolation techniques.

The large T antigen of SV-40 is a virally encoded protein which is synthesized in the cytoplasm but found almost exclusively in the nucleus of infected cells. The primary sequence requirements which allow this localization have recently been defined. Short peptides made up of this sequence have been used to generate antisera against the nuclear localization sequence of the large T antigen and are being used to attempt identification of the molecules involved in translocation of the protein to the nucleus.

2. Mechanisms Regulating Iron Metabolism in Human Erythroleukemic Cells

The regulatory mechanisms which determine the level, locus and affinity of the hepatic receptor for asialoglycoproteins in health and disease states are the subject of study.

The level of asialoglycoprotein receptors on the surface of a human hepatoma cell line (Hep G-2) are effectively modulated when grown in the presence of various desialylated glycoproteins. The loss in the ability of these receptors to bind or to endocytose ligand cannot be accounted for by internalization or by down regulation. No changes were found in the ability of these cells to transcribe or to translate mRNA. Alternate mechanisms are under investigation. Although considerable difficulty has been encountered in obtaining sufficient quantities of human liver necessary to isolate the purified receptor, progress has been made in developing a goat antiserum. Utilizing an IgG fraction from this preparation, provisional evidence has been obtained to indicate that the surface receptors are present but are in a form that renders them incapable of binding and internalizing the appropriate ligand. More exact quantitation is required to confirm the initial findings.

3. Studies on Pathogenesis of Sialic Acid Storage Disease.

Lysosomal transport of sialic acid is being evaluated with respect to its possible relation to the pathogenesis of free sialic acid storage disease.

N-Acetylneuraminic acid (sialic acid) is a 9-carbon acidic monosaccharide serving as terminal residue in a variety of glycoconjugates, e.g., oligosaccharides, glycoproteins and gangliosides. During the course of lysosomal catabolism of such glyco-conjugates, sialic acid is liberated through the action of specific hydrolytic enzymes (sialidases) and passes through the lysosomal membrane to the cytoplasm, where it undergoes further metabolism. In recent years a number of inherited human disorders involving sialic acid has been described which are characterized by the excessive intralysosomal accumulation of free sialic acid. To investigate the possibility that this accumulation is due to defective passage of the monosaccharide across the lysosomal membrane, we have measured the rate of loss of free sialic acid from lysosome-rich granular fractions prepared from cultured fibroblasts of affected patients as well as from normal individuals. Loading of the granular fractions from normal fibroblasts with free sialic acid was achieved by prior incubation of intact cells for two to four days with high concentrations (20-75 mM) of N-acetyl-D-mannosamine (ManNAc), a metabolic precursor of sialic acid. No loss of free, endogenous or ManNAc-derived sialic acid could be detected from granular fractions of mutant cells, whereas rapid loss (half time of 12 min) was observed from the loaded normal granular fractions. These observations support the notion that free sialic acid storage disease is the result of an impaired mechanism of passage of sialic acid across the lysosomal membrane and may provide a second example, human cystinosis being the first, of a lysosomal storage disorder due to defective lysosomal transport.

4. Cell Regulation by the Action of Pharmacodynamic Agents on the Cell Membrane.

The thrust of work in this group is toward an understanding of the mechanisms by which hormonal and pharmacological agents regulate cell activity

and the means by which these mechanisms are subverted by pathologic agents to express themselves in metabolic and digestive diseases. The specific theme centers around the understanding of thyroid physiology, development, and regulation with respect to normal function as well as pathologic states. The structural and functional relationship in the mechanism by which glycoprotein hormones (thyrotropin), certain bacterial toxins (cholera and pertussis), the antiviral protective agent interferon, α-1 adrenergic agents, and insulin and insulin-like growth factors, interact with and transmit their message through the cell membrane to affect thyroid function and pathology, is being further defined. Studies with monoclonal antibodies, based on the idiotype-antiidiotype theory, have continued to explore the importance of these relationships to the expression of thyroid hyperfunction in Graves' disease; to organ-specific autoimmunity in general and to the autoimmunity of Graves' disease in particular; to fluid losses in intestinal diarrhetic states; to thyroid storm and the sympathetic overactivity syndrome of tetanus; to the ability of hormones to modulate the oncogenic state; and to the mechanism by which toxins subvert normal mechanisms to impose their pathological effects. Studies have been continued which evaluate the role of membranes in thyroglobulin biosynthesis and thyroglobulin biodegradation to T3 and T4 and the role of carbohydrate moieties in thyroglobulin structure and post-translational processing. Studies also continue to explore lipid regulation of receptor expression with special emphasis on neuronal and thyroid cell growth and development. Attempts are being initiated to clone hormone and growth factor receptors, important in thyroid regulation of T3/T4 formation, in order to examine their structure and regulatory control at the gene level.

5. Electrochemical Ion Gradients as a Mechanism of Cellular Message Transmission.

The work relates to understanding the biochemical events associated with the normal function of the thyroid and to such pathological conditions of the thyroid, as Graves' disease. Since the previous studies on the effect of thyrotropin on iodide transport, the work has evolved in several directions. (i) Inositol phosphate production has been linked to iodide efflux and to calcium mobilization induced by thyrotropin and norepinephrine through alpha-adrenergic receptor activation. (ii) thyrotropin and norepinephrine-stimulated iodide efflux has been related to the metabolism of arachidonic acid by the lipoxygenase or epoxygenase pathway, and to thyroglobulin iodination and thyroid hormone formation. The work continues to support the hypothesis that alterations in ion fluxes are important early events, as well as primary actions of thyrotropin and pharmacologic agents. The mechanism of iodide fluxes in thyroid cells has been further characterized and the roles of intracellular pH and iodide as regulators of iodide transport have been described. Antiidiotypic antibodies to the thyrotropin receptor have been produced. Preliminary studies suggest that these antibodies can be used to define the spectrum of antibody activities found in patients with Graves' disease, and to determine the molecular structure of the thyrotropin receptor.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 17001-20 LBM

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Mechanisms Regulating Iron Metabolism in Human Erythroleukemic Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Gilbert Ashwell	Institute Scholar	LBM, NIDDK

COOPERATING UNITS *(if any)*

Others: R. D. Klausner	Medical Officer Cell Biology and Metabolism Branch	NICHHD

LAB/BRANCH

Laboratory of Biochemistry and Metabolism

SECTION

Section on Developmental Biology

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The level of asialoglycoprotein receptors on the surface of a human hepatoma cell line (Hep G-2) are effectively modulated when grown in the presence of various desialylated glycoproteins. The loss in the ability of these receptors to bind or to endocytose ligand cannot be accounted for by internalization or by down regulation. No changes were found in the ability of these cells to transcribe or to translate mRNA. Alternate mechanisms are under investigation.

Formerly Z01 AM 17001-19 LBM

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 17002-16 LBM

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Enzymatic Basis of Detoxication

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	William B. Jakoby, Chief Laboratory of Biochemistry and Metabolism		LBM, NIDDK
Others:			
	S. Ansher	Sr. Staff Fellow	LBM, NIDDK
	J. Baker	Sr. NSRA Fellow	LBM, NIDDK
	S. Ramaswamy	Visiting Associate	LBM, NIDDK

COOPERATING UNITS *(if any)*

Peter Cooper University of Kentucky, Department of Medicinal Chemistry
Lexington, Kentucky

LAB/BRANCH

Laboratory of Biochemistry and Metabolism

SECTION

Section on Enzymes and Cellular Biochemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	3	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An investigation is being conducted of those enzymes of detoxication that act on amines. Specifically, two isoenzymes of amine N-methyltransferase from rabbit liver have been obtained which catalyze, with overlapping specificity, the transfer of methyl groups from S-adenosyl-L-methionine to a large number and variety of amines. Serving as methyl acceptors are primary, secondary and tertiary amines of very different carbon skeleton that include aliphatic, aromatic and heterocyclic compounds.

The presence of the enzyme in most animal organs, including brain, suggests the possibility of using pyridine derivatives as prodrugs that could pass the blood-brain barrier. Upon methylation within brain, the resultant pyridinium ion would remain within the cell to exert its pharmacological effect. It should be pointed out that toxic products are also possible: methylation of 4-phenylpyridine results in symptoms of Parkinsonism and methylation of 4,4'-aminodipyridyl yields the toxic paraquat.

Both isoenzymes have been prepared in homogeneous form and have been characterized.

Formerly Z01 AM 17002-15 LBM

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 17003-19 LBM

PERIOD COVERED

October 1, 1985 to September 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Polysaccharides in Morphogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E. Cabib	Senior Research Chemist	LBM, NIDDK
Others:	J. Au-Young	Staff Fellow	LBM, NIDDK
	M.S. Kang	Staff Fellow	LBM, NIDDK
	A. Sburlati	Visiting Fellow	LBM, NIDDK

COOPERATING UNITS *(if any)*

Blair Bowers, Laboratory of Chemistry, NHLBI

LAB/BRANCH

Laboratory of Biochemistry and Metabolism

SECTION

Section on Enzymes and Cellular Biochemistry

INSTITUTE AND LOCATION

NIDDK, LBM, Bethesda, MD, 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	4.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Work on the cloning of the chitin synthetase gene (CHS1) of Saccharomyces cerevisiae , in collaboration with the laboratory of P.W. Robbins, at the Massachusetts Institute of Technology, was continued. It has been confirmed that the cloned gene codes for the synthetase protein. It has also been found that strains harboring a disrupted CHS1 gene are viable and have a normal chitin content. This result suggested the presence of another chitin synthetase, which was subsequently found and characterized.

Studies on the dissociation of fungal β(1→3)glucan synthetase into two fractions, one of which appears to interact with guanosine nucleotides, have been pursued further. The interaction with GTP-γ-S has been demonstrated in different ways. The results have been extended to S. cerevisiae, whose soluble component is now under purification.

Formerly Z01 AM 17003-18 LBM

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 17004-18 LBM

PERIOD COVERED
October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 cherecters or less. Title must fit on one line between the borders.)*
Thermodynamic and Kinetic Studies of Protein Structure and Enzymic Mechanisms

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Peter McPhie Research Chemist LBM, NIDDK

COOPERATING UNITS *(if any)*
Preston Hensley, Associate Professor
Georgetown University (Yeast Arginase)

LAB/BRANCH
Laboratory of Biochemistry and Metabolism

SECTION
Section on Developmental Biology

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	0.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

On Exposure to acid pH, swine pepsinogen undergoes self cleavage to produce psuedo pepsin, by the loss of the first 16 residues in its sequence. Returning to neutrality, produces an irreversible conformational change in psuedo pepsin to a new globular form, which seems to be identical to the rapidly formed transient intermediate previously detected during the folding of intact pepsinogen. Like pepsinogen, but unlike native psuedo pepsin, this neutral pH form of the protein can be reversibly unfolded by urea or high pH. The mechanism of the folding reaction, the structure and stability of this neutral pH form of psuedo pepsin and the role of the first 16 residues in the mechanism of folding of pepsinogen are all under investigation.

Formerly Z01 AM 17004-17 LBM

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 AM 17006-12 LBM

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Expression and Function of Bacterial Cell Surface Components

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: John Foulds, Research Chemist, NIDDK, LBM

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Biochemistry and Metabolism

SECTION
Section on Enzymes and Cellular Biochemistry

INSTITUTE AND LOCATION
NIDDK, LBM, Bethesda, MD, 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Project Number Z01 AM 17006-12 LBM transferred to the Laboratory of Structural Biology.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 17008-03 LBM

PERIOD COVERED
October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Role of the Nuclear Envelope in Intracellular Protein Sorting

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	John H. Hanover	Senior Research Chemist	LBM, NIDDK
Others:	M.K. Park	Visiting Fellow	LBM, NIDDK
	B. Wolff	Guest Workers	LBM, NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Biochemistry and Metabolism

SECTION
Section on Enzymes and Cellular Biology

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	1.3	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The mechanisms which allows localization of proteins to the nucleus and the various organelles of the secretory pathway are under investigation. Using a temperature sensitive mutant of Vesicular Stomatitus Virus (VSV) it is possible to synchronize the intracellular transport of the single glycoprotein of the virus (G protein). The mutant G protein accumulates in the nuclear envelope (NE) and rough endoplasmic reticulum (RER) at 39°C and only proceeds through the secretory pathway at 32°C. We have demonstrated that the NE is not required for transport of G protein. Using an antisera which recognizes only the cytoplasmic domain of this transmembrane protein, the organelles to which it is routed may be purified by immunoaffinity isolation techniques.

The large T antigen of SV-40 is a virally encoded protein which is synthesized in the cytoplasm yet is found almost exclusively in the nucleus of infected cells. The primary sequence requirements which allow this localization have recently been defined. Short peptides made up of this sequence have been used to generate antisera against the nuclear localization sequence of the large T antigen and are being used to attempt to identify the molecules involved in translocation of the protein to the nucleus.

Formerly Z01 AM 17008-02 LBM

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 17009-01 LBM

PERIOD COVERED: October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Tissue Specific and Hormone Regulated Gene Expression

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Lothar Hennighausen	Visiting Associate	LBM, NIDDK
Others:	P. Ghazel	Visiting Fellow	LBM, NIDDK
	H. Lubon	Visiting Fellow	LBM, NIDDK

COOPERATING UNITS *(if any)*

Bernd Groner	Professor	Bern, Switzerland
Bernhard Fleckenstein	Professor	Erlangen, FRG
Jeff Rosen	Professor	Houston, TX

LAB/BRANCH: Laboratory of Biochemistry and Metabolism

SECTION: Section on Developmental Biology

INSTITUTE AND LOCATION: NIDDK, NIH, Bethesda, MD. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.4	3.0	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standerd unreduced type. Do not exceed the space provided.)*

Expression of milk protein genes in the lactating mammary gland is controlled by steroid and peptide hormones. The thrust of work is toward an understanding of the cis-element(s) and trans-acting factor(s) that determine tissue specificity and hormone inducibility of the gene for the whey acidic protein (WAP).

Studies on the interaction of nuclear proteins from mammary epithelial cells with the WAP gene promoter region revealed that the region between -10 and -200 is recognized specifically by at least four different proteins. In order to elucidate the functional significance of those potential trans-acting factors we need to develop in vivo assays.

In collaboration with Dr. Bernd Groner's laboratory (Bern, Switzerland) transgenomic mice were established that carried the Harvey-ras gene under the control of the WAP promoter. The fusion gene was expressed only in the lactating mammary gland. This indicates that the promoter fragment contained the regulatory elements necessary for controlled expression. Furthermore the female mice developed mammary tumors and therefore can be used to study mammary tumorigenesis.

These studies demonstrate for the first time a sequence specific binding of nuclear proteins to a mammary specific promoter and tissue specific expression of this promoter in transgenomic mice.

In the second project we investigate cellular factors necessary to activate human cytomegalovirus, a known pathogen. We started experiments to define in vivo and in vitro activator and repressor elements that govern the expression of the first gene within the viral lifecycle. In collaboration with Dr. Fleckenstein's laboratory we defined sites of protein-DNA interaction in an enhancer and potential repressor element.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 18000-21 LBM (formerly Z01 AM 18000-20 LBM)

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Hormone Dependent Development of Mammary Gland

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Yale J. Topper	Chief, Section on Developmental Biology	LBM, NIDDK
Others:	A. Balapure	Visiting Fellow	LBM, NIDDK
	P. Chomczynski	Visiting Scientist	LBM, NIDDK
	C. Prosser	Visiting Fellow	LBM, NIDDK
	L. Sankaran	Expert	LBM, NIDDK

COOPERATING UNITS *(if any)*

P. K. Qasba — Research Chemist — NCI

Project No. Z01 CB 08218-08

LAB/BRANCH

Laboratory of Biochemistry and Metabolism

SECTION

Section on Developmental Biology

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.5	5.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Estrogen has long been recognized as essential for mammary cell proliferation, but until recently it was assumed that the steroid plays no positive role in milk protein gene expression. However, we demonstrated that estrogen depletion in vivo renders mouse and rat mammary cells virtually incapable of such gene expression in vitro in response to lactogenic hormones, and that this defect can be corrected by administration of estrogen in vivo. The role of estrogen in mammary differentiation has now been further analyzed using the rabbit system. Again, the cells isolated from estrogen-depleted animals have virtually no initial ability to synthesize casein or α-lactalbumin in response to insulin, cortisol and prolactin, although they retain full general responsiveness to prolactin. However, after extended culture in the absence of exogenous estrogen, the cells recover fully their ability of synthesize the milk proteins. By contrast, α-lactalbumin is not synthesized in vivo in the presence of elevated prolactin levels unless estrogen is administered. We interpret the results as follows: 1) A factor(s) which inhibits induction of milk proteins by lactogenic hormones is present in the mammary gland when estrogen is deficient; 2) The putative factor initially present in the isolated, estrogen-deficient mammary tissue is dissipated after extended culture even in the absence of exogenous estrogen; 3) The inhibitory factor(s) remains in the mammary tissue in vivo until estrogen is administered.

Comparison of insulin (I) and IGF-I on mouse mammary cells, in the presence of cortisol and prolactin, shows: 1) Both I and IGF-I, at physiological levels, can induce the glucose transport system up to the level in the 2-day lactating animal. Together, they can induce it to the 10-day lactating level, i.e., the effects are additive; 2) The factors can induce the same maximum level of α-lactalbumin activity; but the specific biological activity of I is 15 times greater than that of IGF-I; 3) I, but not IGF-I, can markedly enhance casein gene expression above the mid-pregnant level.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 18002-13 LBM

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies on the Pathogenesis of Sialic Acid Storage Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Frank Tietze Research Chemist LBM, NIDDK

COOPERATING UNITS *(if any)*

William A. Gahl Research Chemist NICHHD
Section on Human Biochemical and Developmental Genetics

LAB/BRANCH

Laboratory of Biochemistry and Metabolism

SECTION

Section on Developmental Biology

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

N-Acetylneuraminic acid (sialic acid) is a 9-carbon acidic monosaccharide serving as terminal residue in a variety of glyco-conjugates, e.g., oligosaccharides, glycoproteins and gangliosides. During the course of lysosomal catabolism of these glyco-conjugates, sialic acid is liberated through the action of specific hydrolytic enzymes (sialidases) and passes through the lysosomal membrane to the cytoplasm, where it undergoes further metabolic reactions. In recent years a number of inherited human disorders involving sialic acid has been described which are characterized by the excessive intralysosomal accumulation of free sialic acid. To investigate the possibility that this accumulation is due to defective passage of the monosaccharide across the lysosomal membrane, we have measured the rate of loss of free sialic acid from lysosome-rich granular fractions prepared from cultured fibroblasts of affected patients as well as from normal individuals. Loading of normal fibroblast granular fractions with free sialic acid was achieved by prior incubation of intact cells for 2-4 days with high concentrations (20-75 mM) of N-acetyl-D-mannosamine (ManNAc), a metabolic precursor of sialic acid. No loss of free endogenous or ManNAc-derived sialic acid could be detected from granular fractions of mutant cells, whereas rapid loss (t-1/2 ~ 12 min) was observed from the loaded normal granular fractions. These observations support the notion that free sialic acid storage disease is the result of an impaired mechanism of passage of sialic acid across the lysosomal membrane and may provide a second example, human cystinosis being the first, of a lysosomal storage disorder due to defective lysosomal transport.

Formerly Z01 AM 18002-12 LBM

PHS 6040 (Rev 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 18003-13 LBM

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of the Hormone-Dependent Growth and Differentiation of the Mammary Gland

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: T. Oka Senior Investigator NIDDK, LBM

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Biochemistry and Metabolism

SECTION

Section on Intermediary Metabolism

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD, 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Project number Z01 AM 18003-13 LBM transferred to the Laboratory of Molecular and Cellular Biology.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 18004-12 LBM

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biochemical Lesions in Genetic Mucopolysaccharidoses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Irwin G. Leder, Research Chemist NIDDK, LBM

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Biochemistry and Metabolism

SECTION

Section on Intermediary Metabolism

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Project number Z01 AM 18004-12 LBM transferred to the Laboratory of Structural Biology.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 18005-12 LBM

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

B Cell Proliferation: Mechanism of Triggering Activation and Fate of Receptors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Milton Kern, Research Chemist NIDDK, LBM

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Biochemistry and Metabolism

SECTION

Section on Intermediary Metabolism

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Project number Z01 AM 18005-12 LBM transferred to the Laboratory of Chemistry.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 18007-07 LBM

PERIOD COVERED
October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Electrochemical Ion Gradients as a Mechanism of Cellular Message Transmission

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Evelyn F. Grollman	Medical Officer (Research)	LBM, NIDDK
Others:	Elisabeth Bone	Visiting Associate	LBM, NIDDK
	Francesco Alvarez	Guest Researcher	LBM, NIDDK

COOPERATING UNITS *(if any)*

N.J. Philp	University of Pennsylvania School of Medicine
W.A. Valente	University of Maryland
J. Bernar and W.A. Gahl	NIH, NICHHD, Human Genetics Branch

LAB/BRANCH
Laboratory of Biochemistry and Metabolism

SECTION
Section on Cell Regulation

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.9	1.7	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The work relates to understanding the biochemical events associated with the normal function of the thyroid and to pathological conditions of the thyroid, such as Graves' disease. The work continues to support the hypothesis that alterations in ion fluxes are important early events, as well as primary actions of thyrotropin and pharmacologic agents. Since the previous studies on the effect of thyrotropin on iodide transport, the work has evolved in several directions. (i) Inositol phosphate production has been linked to iodide efflux and calcium mobilization induced by thyrotropin and norepinephrine through α adrenergic receptor activation. (ii) Thyrotropin and norepinephrine stimulated iodide efflux has been related to the metabolism of arachidonic acid via the lipoxygenase or epoxygenase pathway; and to thyroglobulin iodination and thyroid hormone formation. The mechanism of iodide fluxes in thyroid cells have been further characterized and the roles of intracellular pH and iodide as regulators of iodide transport has been described. Studies of thyroidal transport have been extended to lysosomal transport of tyrosine and thyroid hormones. These collaborative studies have extended our understanding of the complex metabolism of NaI to thyroglobulin and thyroid hormone release. Antiidiotypic antibodies to the thyrotropin receptor have been produced. Preliminary studies suggest that these antibodies can be used to define the spectrum of antibody activities found in patients with Graves' disease, and to determine the molecular structure of the thyrotropin receptor.

Formerly Z01 AM 23,830-06 LBP

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 18008-20 LBM

PERIOD COVERED
October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cell Regulation by Pharmacodynamic Agents Which Act on the Cell Membrane

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Leonard D. Kohn, M.D.	Medical Director, USPHS, and Chief, Section on Cell Regulation	
Others:	J.Chan	Guest Researcher	LBM, NIDDK
	D. Corda-Luini	Visiting Fellow	LBM, NIDDK
	O. Isozaki	Visiting Fellow	LBM, NIDDK
	C. Taylor	Visiting Fellow	LBM, NIDDK
	P. Santisteban	Guest Researcher	LBM, NIDDK

COOPERATING UNITS *(if any)* E.F. Grollman, H.J.C. Yeh & S. Taylor (All NIDDK): G. Fenzi, A. Pinchera, P. Vitti, & C. Marcocci, (U. Pisa, Sch Med. Italy); R. DeLauro, & E. Consiglio (U. Naples, Italy); R. Toccafondi & C.M. Rotella (U. Florence, Italy); G. Medieros Neto (Clin Endo, SP, Brazil); S.Shifrin (NCI) & W.Gahl (CHHD); W.A. Valente (U. MD, Baltimore)

LAB/BRANCH
Laboratory of Biochemistry and Metabolism

SECTION
Section on Cell Regulation

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.0	6	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Structure-function relationships in the mechanisms by which glycoprotein hormones (thyrotropin), certain bacterial toxins (cholera and pertussis, for example, the anti-viral protective agent, interferon, α_1-adrenergic agents, insulin, and insulin-like growth factors (I and II) interact with and transmit their message through the cell membrane to affect thyroid or fibroblast function and pathology are being further defined. Studies using monoclonal antibodies and the idiotype-antiidiotype theory have continued to explore the importance of these relationships to the expression of thyroid hyperfunction in Graves' disease; to organ-specific autoimmunity in general and the autoimmunity of Graves' disease in particular; to fluid losses in intestinal diarrhetic states; to thyroid storm and the sympathetic overactivity syndrome of tetanus; to the ability of hormones to modulate the oncogenic state; and to the mechanism by which toxins subvert normal mechanisms to impose their pathological effects. Studies have been continued which evaluate the role of membranes in thyroglobulin biosynthesis and thyroglobulin biodegradation to T_3 and T_4 and the role of carbohydrate moieties in thyroglobulin structure and post-translational processing. Studies also continue to explore lipid regulation of receptor expression with special emphasis on neuronal and thyroid cell growth and development. Studies have been initiated to clone hormone and growth factor receptors important in thyroid regulation of T_3/T_4 formation and examine their structure and regulatory control at a gene level.

Formerly Z01 AM 23,960-19 LBP

ANNUAL REPORT OF THE LABORATORY OF CHEMISTRY

NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

SECTION ON BIOCHEMICAL MECHANISMS

TRH ANALOGS

In addition to governing the release of thyrotropin and prolactin in the pituitary gland, TRH (L-pyroglutamyl-L-histidyl-L-proline amide) is known to possess a wide variety of effects on both the central nervous system (CNS) and the cardiovascular system (CVS). TRH has shown promise for use in the treatment of shock, as an analeptic and antidepressant, and as a promoter of the regeneration of injured spinal cord. However, the great variety of its biological effects presents a serious drawback to its use as a specific drug. Our early studies with synthetic analogues of TRH (involving modification of the imidazole ring of histidine) has suggested that the peptide hormone elecits each of its physiological responses at a different receptor and that appropriate analogues may achieve some of the desired specificity of action. In contrast to the natural peptide, 4-fluoro-Im-TRH does not bind to rat pituitary cells in vitro and does not release prolactin from them; such results would immediately suggest the analogue to be nonfunctional. When it is microinjected directly into rat brain, however, it effected significant increases in heart rate and blood pressure. We have now prepared and tested by systemic injection a variety of other analogues on the CVS and endocrine systems of conscious rats. 4-TFM-TRH, 2-TFM-TRH and 4-nitro-TRH were as potent as TRH in increasing mean arterial pressure, pulse pressure and heart rate at both 1 mg/kg and 5 mg/kg doses. At the same time, 4-TFM-TRH and 2-TFM-TRH were 4-5 times more potent than TRH in increasing plasma prolactin; 4-nitro-TRH, on the other hand was totally devoid of prolactin-releasing activity. Nor-Val-TRH was as effective as TRH (at either dose) in increasing plasma prolactin but, surprisingly, was devoid of any CVS activity.

Thus, we have achieved complete separation of these endocrine and CVS effects with 4-nitro-TRH and with nor-Val-TRH. The former compound may be useful in the treatment of shock and the latter as a diagnostic tool for the assessment of pituitary function without the risk of the increased blood pressure and tachycardia induced by TRH. The several imidazole-ring substituents differ in size, electronegativity, polarity, ability to participate in intra- and intermolecular hydrogen bonding, and in hydrophobicity; in the case of nor-Val-TRH, the imidazole ring has been replaced completely by the nonpolar n-propyl group. One or more of these variables may determine the overall conformation of the peptide backbone and the ability of the analogue to bind selectively to a particular receptor. High resolution NMR spectroscopy reveals that the *-CH and ς-CH_2 groups of histidine have different coupling constants in different analogues and, thus, at least the side chain of histidine varies in conformation. Detailed NMR studies are in progress, as well as the syntheses of conformationally rigid analogues of TRH.

HYPOXIC CELL SENSITIZERS

The valuable properties of nitroimidazoles as radiation sensitizers and as selective cytotoxic agents for cancer treatment have stimulated considerable research into mechanisms of action and metabolic fate of the drugs. We have proposed three theories for the mechanism of action: (1) Thiols are known to add to the 4,5-double bond of nitroimidazoles and, thus, such compounds may intefere with normal cellular functions by binding cysteine, glutathione, SH enzymes, etc. (2) Nitorimidazoles may be reduced, *in vivo*, to hydroxylaminoimidazoles which can function as super-nucleophiles in cleaving the phosphate ester bonds of polynucleotides; unfortunately, synthetic hydroxylaminoimidazoles have been found so unstable that their potential as nucleophiles cannot be investigated. As an alternative, we are devising synthetic methods for hydrazimoimidazoles; these compounds should be significantly more stable than hydroxylaminoimi-dazoles and, yet, should possess the same nucleophilic power inherent in hydroxylamine functions. (3) Reduction of the nitro group by nonnucleo-philic agents leads to nitro radicals; we believe these heterocyclic radicals capable of alkylating cell constituents and interfering with metabolism. To this end, we are now studying the reduction of nitroimidazoles with the one-electron transfer agent, titanous chloride.

Misonidazole is an alkylated 2-nitroimidazole which has been found quite effective in sensitizing cancer cells to radiation and in reducing the radiation dose needed to effect significant cell destruction. Unfortunately, the compound has to be used at such high levels as to produce serious side effects and will not be released by FDA. We have postulated that the introduction of nitro groups into more natural imidazoles (histamine, histidine, etc.) may produce the desired alien molecule. Indeed, several such compounds have shown *in vitro* activity comparable to that of misonidazole. Evaluation of the clinical effectiveness in animals of this series of compounds is in progress.

ANTIMALARIALS

Our development, in 1971, of a photochemical route to ring-fluorinated aromatics and heteroaromatics has led to the synthesis of a wide variety of fluoroanalogues of imidazole-based metabolites. Many of these compounds have shown interesting properties as agonists or antagonists and have proved useful as research tools and as possible chemotherapeutic agents. A striking difference has been found between 2-fluoro-L-histidine (2-FHIS) and the 4-fluoro isomer. While the former compound is readily incorporated into new protein in place of histidine (both in bacteria and mammals), the 4-fluoro isomer is not incorporated at all. Furthermore, 2-FHIS shows antibacterial, antiviral, antileukemic and antimalarial properties; again, the 4-fluoro isomer shows none of these activities.

We have become particularly interested in the antimalarial properties of 2-FHIS, since the compound is uniquely and selectively active against *Plasmodium falciparum*, that parasite which is notoriously resistant to chemotherapy. The organism has the unusual property of inducing pro-duction, within an invaded erythrocyte, of a protein containing as much as 70% histidine. The protein is found in "knobs" which are seen on the

erythrocyte surface; these knobs are responsible for a very strong adherence of the infected erythrocytes to capillary endothelium, thereby sequestering parasitized cells which would normally be destroyed during passage through the spleen.

In cultures of infected erythrocytes, low concentrations of 2-FHIS not only inhibit cytoadherence but prevent maturation of the parasite and the appearance of knobs entirely. The assumption that these antiparasitic properties are due to the incorporation of 2-FHIS into the histidine-rich protein is probably unwarranted, since the treated parasite shows a general decrease in protein synthesis and a rather low incorporation of ^{3}H-2-FHIS. As one of several hypotheses for the mechanism of action, we propose that 2-FHIS interferes with histidine as a promoter of the transport of some other amino acids into the cell. This hypothesis is supported by our earlier findings that 2-FHIS inhibits protein synthesis in cell and organ cultures but not in cell-free systems.

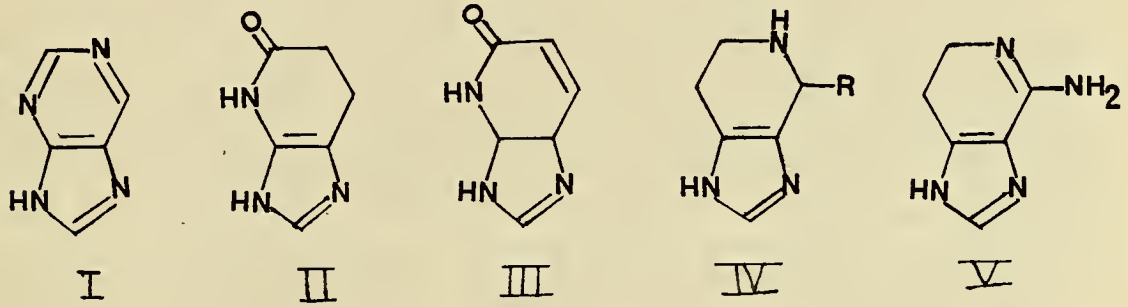
Of some 25 histidine analogues tested thus far, only two show useful antimalarial activity: 2-FHIS is selectively active against P. falciparum, while 2-IHIS shows broad-spectrum antimalarial activity. Surprisingly, neither 2-ClHIS nor 2-BrHIS are active and, thus, 2-FHIS and 2-IHIS may operate by different mechanisms.

Laboratory-scale production of these histidine analogues is extremely time-consuming, involves multiple low-yield steps, and is limited to small batch operation. Our recent efforts to find alternative, and more economical, routes have been successful-at least for 2-IHIS. Readily available 2,4-diiodo-L-histidine can be converted into mixtures of 2-IHIS, 4-IHIS and HIS by photoreduction, catalytic hydrogenation or reduction with titanium trichloride. The last method is especially promising, providing yields of 2-IHIS up to 20% in this one-step process. Since both 4-IHIS and HIS are innocuous, the total reaction mixture may be of therapeutic use without further fractionation. More recently, we have found that 2,4-diiodo-L-histidine can be reduced selectively with hot 3N HCl to 2-iodo-L-histidine, without formation of any of the 4-iodo isomer. While both the 2-fluoro and 2-iodo analogues show high antimalarial activity in vitro, tests with monkeys show the 2-fluoro compound to be too toxic and the 2-iodo compound to be inactive. It is possible that mammals possess a metabolic system for deiodination of the iodo analog. Deiodination of 4-iodohistidine in rats had been observed previously. In the initial in vitro screening, 2-azidohistidine was also found to have some activity against P. falciparum. It is possible, therefore, that the size of the 2-substituent is critical; we are now devising synthetic routes to other 2-substituted histidines with appropriate size but with greater resistance to metabolic breakdown.

Clues to the design of effective antimalarials may be achieved from knowledge of the mechanisms of action of these histidine analogues. To this end, a synthesis of ^{14}C-2-flurorohistidine has been developed. ^{3}H-2-iodohistidine is being prepared by selective reduction of 2,4-diiodohistidine with ^{3}HCl.

IMIDAZOLE ANTIVIRALS

The notable success of virazole and deazapurine systems as antivirals has stimulated research into further modifications of the purine (1) ring system, especially those involving replacement of ring nitrogen with carbon. Analogues synthesized to date have required laborious multistep processes and have given only low yields. We have devised a number of simple syntheses which produce deazapurine analogues in good yield. Reduction of 4-nitrohistidine ester or of 4-nitroimidazole propionic ester leads to II.



Reduction of 4-nitrourocanic ester gives the stable 4-aminourocanic ester, but subsequent irradiation converts the trans olefin to cis and the product cyclizes to III. Condensation of histamine with aldehydes gives series IV. Finally, cyclization of 4-(trifluoromethyl)histamine with ammonia gives V. Each of these systems can be dehydrogenated to the fully aromatic system with selenium dioxide. These compounds, with or without ribose attachment, will be evaluated for anitviral activity.

NEW HISTIDINE AND HISTAMINE ANALOGUES

In the preceeding sections, we have described a variety of significant and valuable applications of histidine analogues in biochemical and pharmacological studies. Such studies could have been performed many years ago, but for the fact that the analogues had not been available through classical or obvious synthetic routes. In order to extend our studies and find new analogues which are of possible clinical use, we need to develop even more novel synthetic methods. We have developed procedures for the conversion of aminohistidine into azido and nitrohistidine, of amino to chloro, bromo and iodo, of trifluoromethyl to methyl and cyano, etc. Recently, we synthesized 2- and 4-(pentafluoroethyl)-histidines by photochemical radical substitution. These compounds are converted by base into the corresponding (trifluoroacetyl)histidines, which have such reactive carbonyl groups that they may serve as affinity labels for histidine-binding

sites. Upon treatment with methanolic base, (trifluoromethyl)histidine can be converted into trimethoxymethyl)histidine and pentafluoroethyl into the corresponding ketal. These ortho functionalities are also of interest as potential convalent affinity labels. Studies are in progress for synthetic routes to alkyl, alkoxy, aryl, formyl and phosphonohistidines and histamines.

CHEMISTRY OF SUBSTITUTED IMIDAZOLES

Ring-trifluoromethylated imidazoles show the unique property of losing hydrogen fluroride above pH8 to form metastable difluoroduzafulvenes, which then react with any available nucleophile to form new covalent bonds. Such intermediates, derived from trifluoromethylhistamine or histidine, may be able to serve as covalent affinity labels for specific binding sites, both in vitro in vivo. It would be desirable, therefore, to have available a series of trifluoromethyl analogues with a range of reactivities, and to be able to correlate reactivity with some substituent parameter. Our discovery of a simple photochemical method for the trifluoromethylation of imidazoles has made available a large series of analogues for study. We have now found that the reactivities of some members of the group can be correlated with the special electronic effects of certain substituents (capable of hyperconjugation or back-bonding). Computer analysis of reactivity data for a series of trifluoromethylimidazoles has provided a linear free energy relationship in which log k_r correlated with both inductive and resonance components of the respective substituents. According to computer-based predictions, the fluoro group would provide the ideal combination of acidity and reactivity under physiological conditions. We have, therefore, developed procedures for sequential photochemical introduction of fluorine and trifluoromethyl into imidazoles and have verified the predicted reactivities. We are now involved in the preparation of peptide hormones containing these substituents. Photochemical introduction of the trifluoromethyl group has been found practical for more complex imidazoles and studies are under way for the synthesis of the trifluoromethyl analogue of the anti-ulcer drug, cimetidine.

CHEMISTRY, BIOCHEMISTRY AND PHARMACOLOGY OF BIOINDOLE ANALOGS:

Tryptophan is an essential amino acid, serving as the precursor of the neurotransmitter, serotonin, and of the hormone, melatonin, in addition to its roles in enzymes and in receptor proteins. Tryptophan is metabolized in mammals by a pyrroloxygenase in the liver, where it can serve as a precursor of nicotinamide (Vitamin B_6) in some animals. In other tissues, tryptophan and related indoles are metabolized by a distinct oxygenase, the activity of which is dramatically increased (up to 100-fold) upon administration of bacterial lipopolysaccharides or interferon. The role of this oxygenase in the response of the organism to infection is unknown, however. We anticipated that certain 2-substituted tryptophans might serve as selective "suicide substrates" for these oxygenases. Analogs of trypto-

phan with electronegative substituents at C-2 had not been previously prepared. We have obtained 2-chloro and 2-bromo-L-tryptophan by radical halogenation, 2-trifluoromethyl-L-tryptophan by photochemical substitution, and 2-nitro-L-tryptophan as a minor product of direct nitration. Both the trifluoromethyl and nitro groups can be converted readily into other functions; some of these derivatives are of potential value as affinity and photoaffinity labels, as antibacterial agents and as photosensitizers in radiation therapy. 5-Azido-L-tryptophan has already been found effective as a photoaffinity label for tryptophan synthase.

The mechanisms of hydrolysis of the 2-halotryptophans at low pH have now been fully elucidated and reveal the involvement of intramolecular proton transfer in the conversion of the stable indole to the labile indolenine tautomer. An enzyme carboxyl group should also promote indolenine formation, suggesting the indolenine to be the true substrate for certain tryptophan enzymes.

The first conclusive support for this concept is found in the demonstration that 2,3-dihydro-L-tryptophan and oxindolyl-L-alanine, analogs of the indolenine tautomer of tryptophan (tetrahedral carbon at C-3), are potent competitive inhibitors of tryptophan synthase and tryptophanase. Furthermore, the two enzymes show opposing specificity for the C-3 diastereoisomers of 2,3-dihydro-L-tryptophan, suggesting that these enzymes catalyze their reactions via enantiomeric indolenine intermediates.

Fluorine-19 nuclear magnetic resonance and differential absorption spectroscopy have been used to study the binding and reactions of the D and L isomers of 5-fluorotryptophan, tryptophan and of (3S)- and (3R)-2,3-dihydro- 5-fluorotryptophan. Tryptophan synthase specifically and tightly binds the (3S) diastereoisomer of both 2,3-dihydro-5-fluoro-D-tryptophan and 2,3-dihydro-5-fluoro-L-tryptophan, whereas it binds 5-fluoro-D-tryptophan more tightly than 5-fluoro-L-tryptophan. Unexpectedly, we find that the D and L isomers of 5-fluorotryptophan, tryptophan, and (3S)-2,3-dihydro-5-fluorotryptophan are slowly interconverted by isomerization reactions. These isomerization reactions are much slower than the β-replacement and β-elimination reactions catalyzed by tryptophan synthase. Since pyridoxal phosphate itself slowly catalyzes many reactions of amino acids in model systems, our results raise the interesting question of whether tryptophan synthase itself serves a catalytic role in these slow reactions or whether the enzyme simply binds the substrate and pyridoxal phosphate stereospecifically and thus promotes the intrinsic catalytic activity of pyridoxal phosphate. Our results further define the stereochemistry of the substrate binding site of tryptophan synthase.

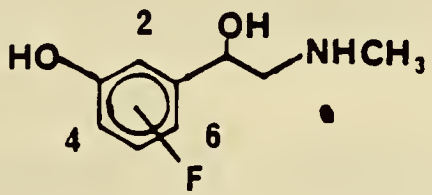
GENERAL PRINCIPLES OF ENZYME CATALYSIS AND SIMULATION:

In order to account for the remarkable catalytic power of enzymes, it is generally considered that the activation free energy (the energy hill which must be surmounted to get from starting material to product) is contributed both by binding of the substrate to the enzyme (step 1) and by chemical manipulation of the bound substrate (bond-making and breaking, step 2). Popular opinion holds that most of the activation energy is supplied in step 2: We have proposed, however, that the overall catalytic process can be explained more reasonably if it is assumed that the first step (binding) contributes a more significant and sometimes major, share of the activation energy. To support this theory, we have synthesized a large variety of test-tube models which simulate the bound substrate by being frozen into a single, favorable conformation and by having the interacting groups brought into the closest possible juxtaposition (stereopopulation control). The compounds undergo intramolecular reactions at rates comparable to those catalyzed by enzymes, sometimes even too fast to measure. Enzymes catalyze many reactions which cannot be observed under mild laboratory conditions. We have shown that our "locked" test-tube analogs can undergo a number of these reactions under physiological conditions of temperature and pH. Thus, one can demonstrate such difficult processes as hydride transfer and displacement of aromatic halogens.

Agonist Properties of Fluorinated Biogenic Amines

Using 6-fluoronorepinephrine (6FNE) and 2-Fluoronorepinephrine (2FNE) as specific alpha- and beta-adrenergic agonists, respectively, the effects of repeated restraint stress, of adrenocortotropin (ACTH), and of desmethylimipramine (DMI) on the alpha- and beta-adrenergic components of the cAMP response to catecholamines in rat brain slices were measured. From this study, the conclusion was reached that restrain stress acts primarily to reduce the response to stimulation of central alpha-adrenergic receptors while DMI acts primarily to reduce the response to stimulation of beta-adrenergic receptors. ACTH has the same effect as restraint stress, suggesting that pituitary hormones mediate the stress effect [K. Kirk, E. Stone (NYU)].

The pharmacological profiles of fluorinated phenylephrines, 1a-b (FPE), have confirmed that fluorine in the 6-position of PE increases potency at both alpha-1 (displacement of [3H]WB-4101 and [3H]prazocine in brain membranes, stimulation of phosphatidylinositol turnover and augmentation of 2-chloroadenosine stimulation of cAMP accumulation in brain synaptoneurosomes and induction of contraction of aortic strips), and alpha-2 (displacement of [3H]clonidine in brain membranes and inhibition of stimulated adenylate cyclase in human platelet membranes) adrenergic receptors and decreases potency at beta adrenergic receptors, 6FPE has an alpha-1/beta receptor selectivity of 86-140 fold while PE has a selectivity of only 2-5 fold. The alpha-2/beta receptor selectivity for 6FPE is 780 fold, compared to a 33 fold selectivity for PE.



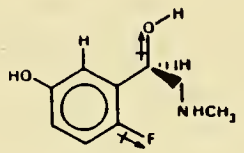
1a-b

2FPE has slightly lowered potency at alpha adrenergic receptors, and significantly increased potency at beta-1 and beta-2 adrenergic receptors. Indeed, 2FPE is somewhat selective for beta-adrenergic receptors compared to alpha-1 adrenergic receptors and is only 3-fold more potent at alpha-2 adrenergic receptors than at beta-receptors.

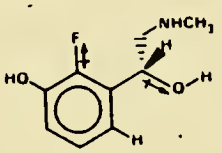
4FPE showed the least effects of the presence of fluorine in the ring, being slightly less potent in all systems than PE [F. Gusofsky, J. Daly, C. R. Creveling (LBC), and K. Kirk].

The increased potency seen with 6FPE has prompted us to develop ring fluorinated analogs of another important N-methylated amine, epinephrine [A. Adejare, K. Kirk].

In addition to the practical implications of the development of a more potent and more selective alpha-adrenergic agonist, the discovery of increased potency due to fluorine substitution is unprecedented and has important theoretical implications. Thus, our previous model to explain the effects of fluorinated substitution on adrenergic properties invoked inhibition of binding--i.e., a purely negative effect. Increased potency clearly is not consonant with such a model. We now suggest that conformational destabilization caused by dipole-dipole repulsion, shown in Fig. 1, favors rotameric structures favorable to binding to alpha- or beta-adrenergic receptors.

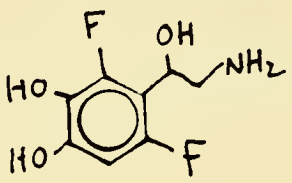


R-6 FPE
α-ADRENERGIC CONFORMATION



R-2 FPE
β-ADRENERGIC CONFORMATION

Progress in projects designed to test experimentally this model is summarized below. First analogs are being synthesized which have fluorine substituted in both of the critical positions, viz., 2,6-di-FNE (2) and 2,6-di-FPE (3) [G. Chen, K. Kirk].



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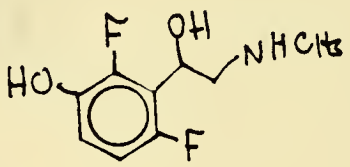
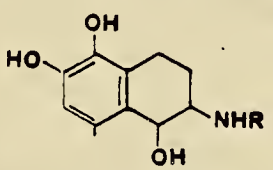


3

The adrenergic properties of these two analogs should give important information regarding the role fluorine plays in determining adrenergic specificities.

In a second approach, conformations of adrenergic agonists can be fixed by including the ethanolamine side-chain in a ring. Such is the case of the trans-beta-aminotetraol 4, a potent beta-adrenergic agonist. The ring-fluorinated analog 5 represents a compound with two opposing conformational changes (directly determined in this semi-rigid analog) should provide valuable evidence for the validity of our new model.



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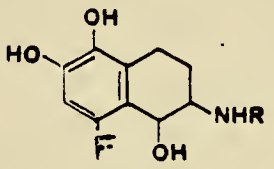


5

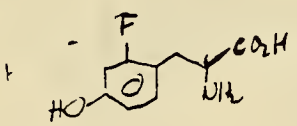
Fluorinated Analogs as Biological Tracers

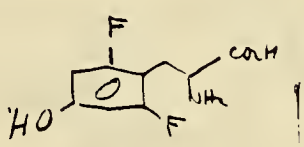
Two chemo-enzymatic routes have been used to synthesize radio--labelled 6F-L-DOPA. In the first of these, a cinnamic acid derivative was reduced with tritium gas. Selective deacylation of the S-enantiomer with acyalase followed by BBr3 demethylation gave ^{3}H-6F-L-DOPA [D. Furlano, K. Kirk].

In a second approach, we have shown that 6-F-L-DOPA is efficiently synthesized from 4-fluorocatechol and pyruvic acid using tyrosine phenol lyase to catalyse the coupling. The use of [14C]pyruvate directly produces ^{14}C-6F-L-DOPA [R. Phillips (U. of Ga.), D. Furlano, K. Kirk].

Fluorinated Analogs as Prodrugs

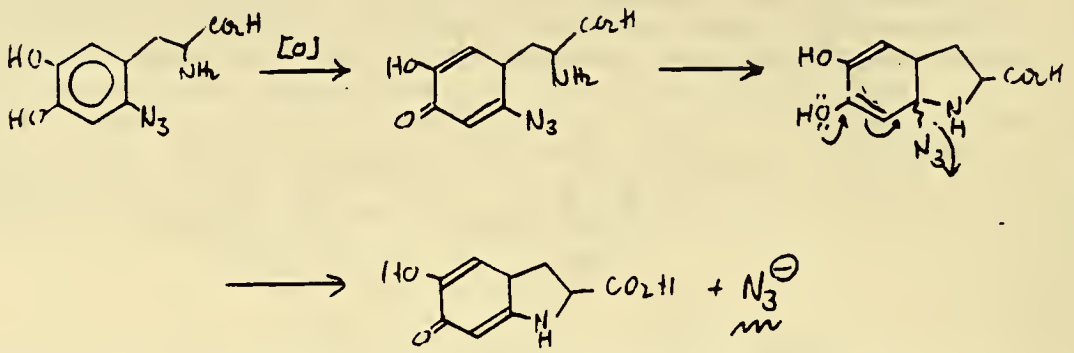
Despite the impressive selective toxicity of 6F-D,L-DOPA towards human melanoma cells in vitro, only marginal in vivo effects were observed. Since a tyrosine precursor to DOPA could be a better substrate for uptake into melanoma cells, and since this amino acid would not be subject to rapid metabolism by COMT as is DOPA and its derivatives, we have synthesized the fluorinated analogs 6 and 7 and have submitted them for evaluation against melanoma cell lines.





Because of the extreme toxicity of azide ion to cells, we have explored syntheses of azido-substituted amino acids which would be capable of releasing azide ion during tyrosinase catalyzed oxidation in vivo (scheme 7). Tyrosine phenol lyase catalyzed the synthesis of 2-azidotyrosine in excellent yield from 3-azidophenol and pyruvic acid. 4-azidocatechol has been synthesized and will be used in a similar

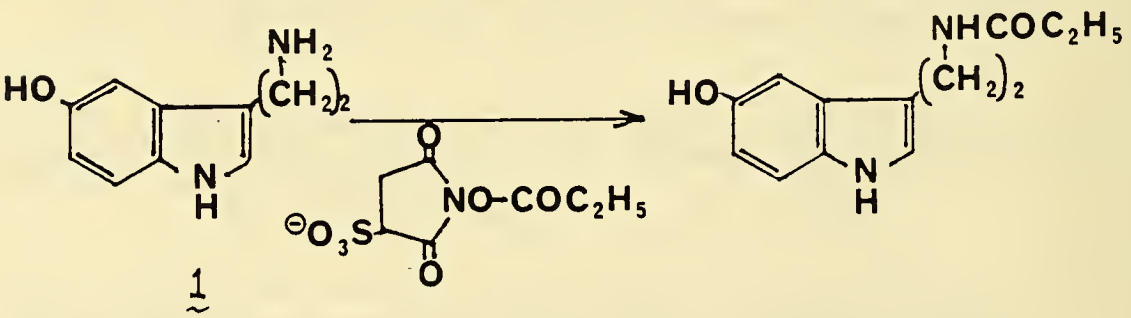
synthesis. These compounds will be evaluated as anti-melanoma drugs [R. Phillips, D. Furlano, K. Kirk].



Development of Sensitive and Efficient Methods for the Detection of Biogenic Amines and Their Metabolites in Biological Samples

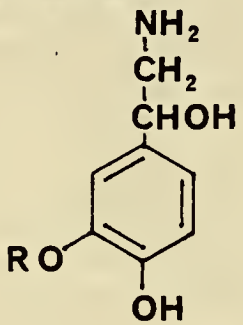
We have reported that N-hydroxysuccinimide esters readily acylate trace quantites of amines, including neurotransmitters in CSF, in high yield. A simple and selective acylation of the amino group results in a less polar derivative, which may be extracted into an organic phase or otherwise separated from ionic species in the biological sample. In cases where the native amine is not sufficiently electroactive, a similar acylation using an N-hydroxysuccinimide ester of an electroactive carboxylic acid leads to a derivative which may be detected electrochemically.

The strategy of acylation with simple acyl group (N-propionyl) to facilitate isolation and concentration was used to develop an extremely accurate method for the assay of 5-hydroxytryptamine (5HT), with a sensitivity of 20 parts per trillion. Clinical applications of this method have included direct measurement of 5HT levels in patients suffering varying levels of depression and in arsonists. Preliminary results suggest that elevated levels of 5HT may be involved in certain clinical disease states.



1

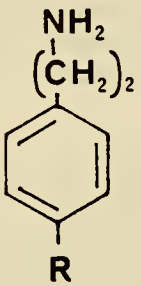
A major current interest of normetanephrine (NMN, 2) is in its relationship to the monamine hypothesis of effective disorders. As a major metabolite of norepinephrine (NE)-- an intrasynaptic deficit of, NE is postulated to be a causative factor in affective disorders--the accurate measurement of CSF NMN is critical in the study of NE turnover as a function of disease states and drug treatment. We have now used our strategy of derivitization to develop an extremely accurate and simple NMN assay and have demonstrated its clinical utility by the determination of CSF levels of NMN in patients suffering from alcoholism. 3-Ethoxy-4-hydroxyphenethanolamine (EHPEA, 3) was used as an internal standard in this assay. The N-propionyl derivatives of NMN and EHPEA have comparable retention times but are clearly separated by reverse phase HPLC. Using this internal standard, the analytical procedure permitted a practical limit of sensitivity of below 0.025 pMol per mL of CSF. Using this method a group of drug-free, hospitalized alcoholic patients were shown to have levels of CSF NMN in the 0.5-1.5 pMol per mL range. Combined with methods for measuring CSF HVA and NE, this procedure will greatly facilitate the assessment of the role of NE in affective disorders.



$R = CH_3$ 2

$= C_2H_5$ 3

Phenethylamine (PE4) is an endogenous amine present in different mammalian tissues, including the brain. When injected into animals, PEA exerts stimulatory effects similar to amphetamine. Changes in endogenous levels of PEA have been implicated in the etiology of certain psychiatric disorders. Previous methods for quantitation of PEA have relied on GC-MS, a technique requiring expensive instrumentation not generally available in clinical laboratories.



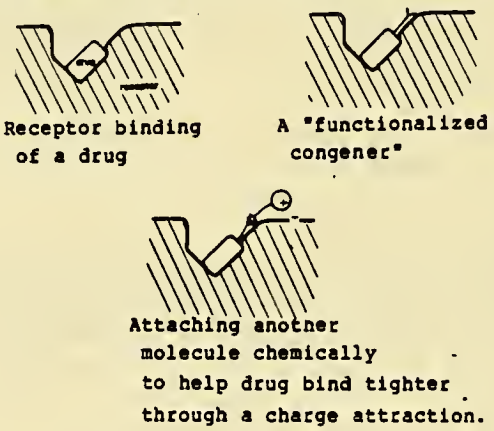
$R = H$ 4

$= CH_3$ 5

Since PEA is not readily oxidized by electrochemical oxidation, in order to use HPLC coupled with electrochemical detection we have again used the dual strategy of acylation of this amine with the electroactive acylating reagent described in our histamine assay. The procedure consists of the reaction of PEA with sulfosuccinimidyl 3(4-hydroxyphenyl) propionate. The resulting derivative is readily extracted and concentrated for HPLC analysis. The assay is very sensitive (10 pg) and has been used to measure PEA levels in non-human primate CSF. In this assay, R-tolylphenethylamine, 5, was used as the internal standard [M. Linnoila (NIAA), F. Gusofsky, K. Jacobson, K. Kirk].

Functionalized Congeners of Bioactive Compounds:

By the functionalized congener approach to drug design, new analogs are synthesized with the regiospecific inclusion of a functionalized chain at a point which can accommodate molecular modification and a certain degree of steric bulk. The resulting functionalized drug congener may then be attached through an amine or other reactive group on the chain to various organic moieties, such as amines and peptides. The receptor-binding affinity of these analogs is often greater than that of the parent drug and does not necessarily diminish as the molecular weight is systematically increased. The concept of utilizing distal sites of interaction to enhance the affinityof a drug at its receptor is illustrated in the following figure:



Applications of the functionalized congener approach include probes for receptor studies and improved drug delivery (including targeting and altering the characteristics of passage through membranes). Drug conjugates may be designed in a stepwise approach to optimize certain pharmacological properties, such as potency, specificity, and duration of action.

Adenosine acts as a neuromodulator in the circulatory, endocrine, immune and central nervous system. The biological activity is associated with two receptor subtypes: the A_1-adenosine receptor mediates cardiac and central depressant and antilipolytic activities and is coupled to adenylate cyclase in an inhibitory manner; the A_2-receptor is involved in vasodilation and antithrombotic functions, possibly

through stimulation of adenylate cyclase. The major class of antagonists, the alkylxanthines, generally acts at both receptor subtypes; there is currently a search for analogs which have both high potency receptor subtype selectivity and water solubility.

We have developed a series of functionalized congeners, based on N^6-phenyladenosine, as agonists at adenosine receptors. N^6-[p-(Carboxymethyl)phenyl]adenosine was synthesized as a functionalized congener. This carboxylic acid congener retained biological activity, and the carboxylate group could then be coupled to various alkyl and aryl amines using carbodiimide reagents with the aid of the catalytic additive N-hydroxybenzotriazole. It was found that aryl amides at this position tended to be more potent receptor ligands than alkyl amides, thus an additional spacer group consisting of p-aminophenylacetic acid was added (as in 2). In each case, potency depended on the nature of the spacer group and the terminal moiety. A particularly potent congener (3, n=2) contained a terminal 2-aminoethylamide moiety and had a K_i value of 0.85 nM in competitive binding assay against [^{3}H]N^6-cyclohexyladenosine (CHA) on rat cerebral cortex membranes (A_1-receptor). This amino congener could be acylated readily using active esters such as N-hydroxysuccinimide ester, to give extended amides. The reactions products were analyzed using californium-252 plasma desorption mass spectrometry, since standard methods of mass spectrometry could not be used due to the high molecular weights. [K. Jacobson, K. Kirk, J. Daly, & H. Fales]

Protein conjugates of adenosine linked through the biotin-avidin complex have been synthesized and indicate that A_1-adenosine receptor binding still occurs with analogs having molecular weights in excess of 100,000 Daltons. These conjugates are potentially useful for affinity chromatographic isolation of the adenosine receptor or for histochemical studies. The adenosine-biotin conjugates bound to avidin with the expected 4:1 stoichiometry. Alone it bound to the A_1-adenosine receptor with an affinity constant of 11 nM. After preincubating the adenosine analog with a large excess of avidin, the affinity at the adenosine receptor was decreased by a factor of only 3. Such avidin conjugates bound competitively to the A_1-adenosine receptor even when the avidin was conjugated to fluorescent or enzymatic probes. Similar bifunctional conjugates consisting of biotin coupled to a xanthine congener required a larger spacer chain in order to bind simultaneously to both avidin and the A_1-adenosine receptor [K. Jacobson, K. Kirk, and J. Daly].

Adenosine analogues substituted at N^6 with spacer arms designed for attachment to soluble macromolecules or to solid supports were evaluated as agonists at the A_2-adenosine receptor that mediates coronary vasodilation in the dog. The most active analogues had spacer arms terminating in $-NH_2$, $-NHCH_3$ or in a biotin residue. Comparisons of coronary vasoactivity with affinity for brain A_1 adenosine receptors identified one biotin-containing analogue as relatively selective for coronary A_2 receptors. The complex of this analogue with avidin retained coronary vasoactivity. Thus, these functionalized congeners of adenosine are potentially useful for the isolation by affinity chromatography of the A_2- as well as the A_1-adenosine receptor [K. Jacobson, R. Olsson, K. Kirk, G. Stiles, and J. Daly].

Caffeine (1,3,7-trimethylxanthine) and the closely related theophylline (1,3-dimethyl-) act as antagonists at extracellular adenosine receptors. The xanthines are used clinically as central stimulants, respiratory stimulants, cardiac stimulants, antiasthmatics and have diuretic action. These drugs have associated with them the problems of side effects due to multiple sites and mechanisms of action. Their use is often limited by cardiac effects, and at very high doses the xanthines cause convulsions. Therefore, the xanthines represent a challenge to the medicinal chemist in finding ways to increase the specificity of action.

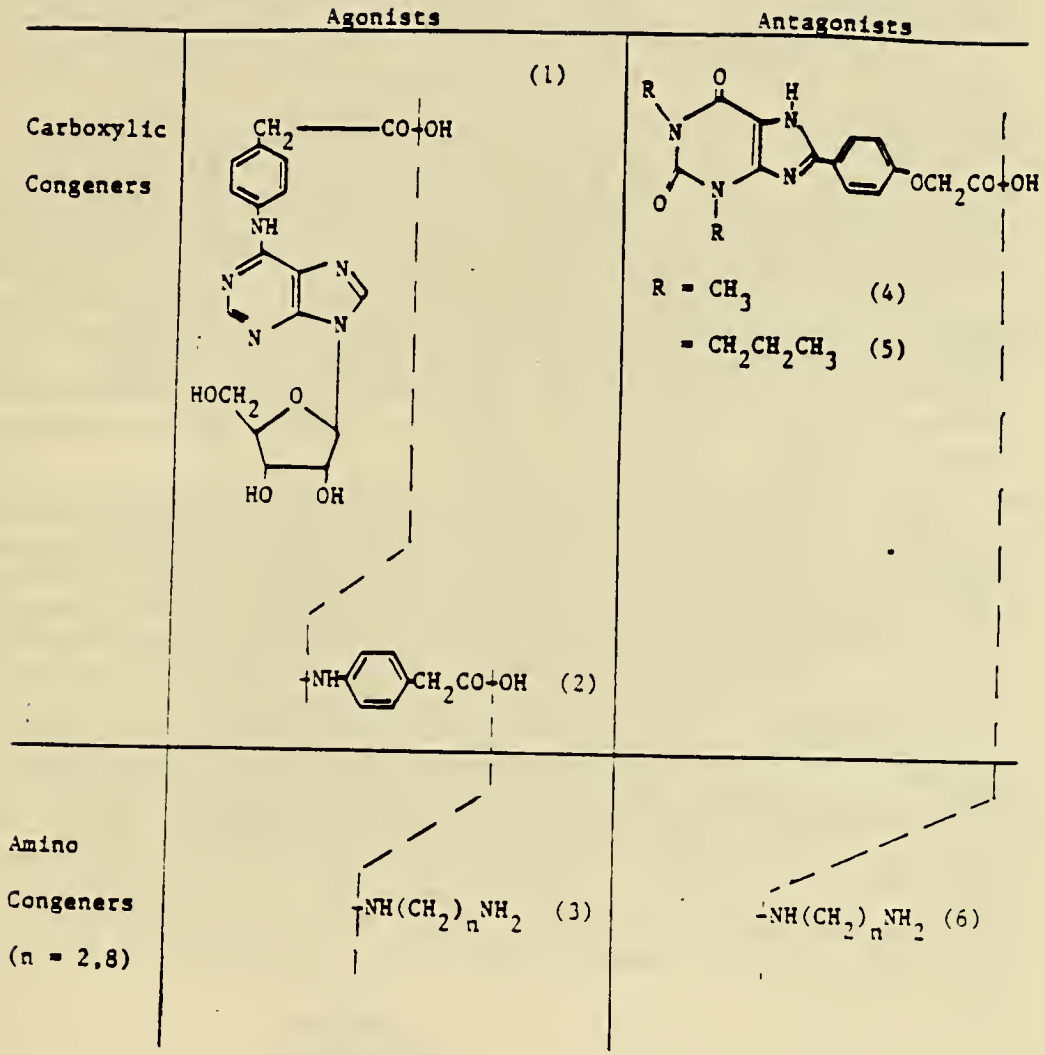
In the series of antagonists, 8-[p-carboxymethyloxyphenyl] derivatives (4 and 5) of theophylline and of 1,3-dipropylxanthine, as well as many related analogs, were synthesized as functionalized congeners. The carboxylic acid group may be coupled to small amines or to amines on larger carriers without abolishing receptor binding. Thus we have identified this position of modification as being relatively insensitive to the presence of large groups. Potencies at the A_1-receptor included K_i values of 1.2 nM (10^4X more potent than theophylline) for a congener with a terminal 2-aminoethylamide moiety (6, n=2, R=Pr) and 58 nM for the parent carboxylic acid (5). The high potency of amino congeners in the antagonist series parallels effects of amino groups on the attached chain finding in the agonist series.

The changes in activity resulting from different charged groups on the chain confirmed that the activity of the drug could be modulated through distal structural changes. The attachment of functionalized congeners to increasingly larger carriers did not necessarily diminish the potency at the adenosine receptor. The unusual effects of charged groups at the end of the chain suggested the synthesis of a series of amino acid and peptide conjugates of the xanthines. In addition to retaining the carboxylic or amino group, which seemed to be related to the receptor subtype specificity, a wide variety of side chains were introduced to explore the limits of what groups are tolerated at the receptor binding site.

Once again the trend was observed that an amino group on the end of the chain was associated with high A_1-receptor potency and selectivity. Amino acid residues of the D-configuration and a wide variety of side chains were tolerated, thus confirming that we had created a functionalized site on the molecule with relaxed steric and chemical restrictions for potency at the receptor. Thus this promised to be a general site for attachment of probes for studying receptors or vectors for targeting drugs. A D-lysyl conjugate displayed a water solubility of 340 micromolar and an affinity constant at the A_1-receptor of 0.87 ± 0.09 nM. [K. Jacobson, K. Kirk, & J. Daly]

Amino acid and peptide derivatives derived from 1,3-dipropyl-8-(p-carboxymethyloxyphenyl)xanthine, have been investigated as antagonists at A_2 adenosine receptors stimulatory to adenylate cyclase in membranes from rat pheochromocytoma PC 12 cells and human platelets and at A_1 adenosine receptors inhibitory to adenylate cyclase from rat fat cells. The functionalized congeners and conjugates have affinity constants ranging from 80 to 310 nM at A_2 receptors of PC 12 cells and from 25 to

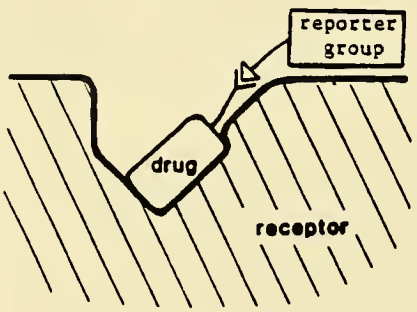
Figure. Functionalized congeners of adenosine receptor ligands.



135 nM at those of platelets. The affinity of the xanthine derivatives at A_1 receptors of fat cell are in the 15 to 30 nM range. Thus, the amino acid and peptide conjugates have high potencies at both receptor subclasses and show some selectivity toward A_1 adenosine receptors. Derivatives of the congeners should be useful as receptor probes and as radioiodinated ligands [D. Ukena, J. Daly, K. Kirk, K. Jacobson].

Compound 6 (n=2, R=Pr) (XAC), an amine functionalized congener was prepared in a tritiated form starting with a 1,3-diallyl precursor. [^{3}H]XAC has higher receptor affinity (K_D 1.2 nM in rat brain and 0.17 nM in calf brain at 37°C), higher specific activity (158 Ci/mmol), lower non-specific membrane binding, and more favorable hydrophilicity than [^{3}H]1,3-diethyl-8-phenylxanthine (DPX). Adenosine agonists and antagonists compete for [^{3}H]XAC binding sites in brain in an order of potency characteristic of A_1 adenosine receptors. Although XAC is at least one order of magnitude selective for A_1 adenosine receptors, [^{3}H]XAC is also useful as a radioligand for A_2 adenosine receptors. It binds to human platelet membranes with a K_D of 12 nM at 37°C. The potencies of adenosine agonists and antagonists in inhibiting [^{3}H]XAC binding to platelet membranes are commensurate with their potencies at A_2 receptors. [^{3}H]XAC is, therefore, the first truly satisfactory antagonist radioligand for adenosine receptors. In addition to binding assays in tissue homogenates it is now being used for the autoradiograhic localization of adenosine receptors in the brain and in peripheral organis, such as the heart and kidneys [K. Jacobson, D. Ukena, K. Kirk, M. Williams, J. Daly].

The "functionalized congener" approach has been extended to the synthesis of spectroscopic and other probes for adenosine receptors that retain high affinity (10^{-9} -10^{-8} M) in A_1-receptor binding. The probes have been synthesized from an antagonist xanthine amine congener (XAC) and an adenosine amine congener (ADAC). [^{3}H]ADAC has been synthesized and found to bind highly specifically to A_1-adenosine receptors of rat and calf cerebral cortical membranes with K_D-values of 1.4 nM and 0.34 nM, respectively. The higher affinity in the bovine brain, seen also with many of the probes derived from ADAC and XAC, is associated with phenyl substituents. The spectoscopic probes contain a reporter group attached at a distal site of the functionalized chain. These bifunctional ligands may contain a spin label (eg. the nitroxyl radical TEMPO) for electron spin resonance spectroscopy, or a fluorescent dye, including fluorescein and 4-nitro-benz-2-oxa-1,3-diazole (NBD), or labels for ^{19}F nuclear magnetic resonance spectroscopy [K. Jacobson, K. Kirk, J. Daly].



The ability of caffeine, enprofylline (3-propylxanthine), 8-phenyltheophylline, 8-p-sulphophenyltheophylline, 8-(4'-carboxymethyloxyphenyl)-1,3-dipropylxanthine (compound 5) and 8-(4'-carboxymethyloxyphenyl)-1,3-dipropylxanthine 2-aminoethylamide (compound 6) to antagonize the effects of a potent adenosine agonist, N-5'-ethylcarboxamidoadenosine (NECA), on heart rate and blood pressure in anesthetized rats was examined. The first five xanthine derivatives were equally active in antagonizing the two responses. By contrast, compound 3 was 16 times more potent in antagonizing the heart rate response than the blood pressure response. Since adenosine reduces heart rate via an effect on A_1-receptors and the blood pressure effect is mediated via A_2-receptors the results suggest that compound 6 is a selective peripherally active A_1-adenosine receptor antagonist *in vivo*.

Furthermore, measurements of the concentration of the compound 3 in plasma and brain indicate that it penetrates poorly into the CNS. This compound thus has potential as a very valuable research tool for such applications as elucidating which adenosine actions are mediated via peripheral and which via central adenosine receptors. Other applications would include the defining of the relative importance of A_1- and A_2-adenosine receptors in various peripheral tissues, including kidney and lung. A compound of this type could have clinical potential as an inotropic and diuretic agent without the side effects in the central nervous system.

The *in vivo* persistence of compounds 5 and 6 (Table 2) is comparable to half-life values reported for theophylline. Thus, neither the amide bond nor the ether linkage to the para-position chain of the 8-phenyl ring is highly sensitive to *in vivo* cleavage. This is an important consideration in the design of more complex conjugates in which the primary pharmacophore is intended to remain attached to the "carrier". [K. Jacobson, B. Fredholm, K. Kirk, J. Daly]

Adenosine agonists of the A_1-subclass produce renal vasoconstriction *in vivo* and *in vitro*. Xanthines such as theophylline reverse this effect. The A_1-selective antagonist XAC was 2500 times more potent than theophylline in reversing kidney vasoconstriction in isolated rat kidneys [P. Churchill and K. Jacobson].

SECTION ON CARBOHYDRATES

This section continues the study of the interaction of antigens with (monoclonal) antibodies on the molecular level. The approach is two-fold.

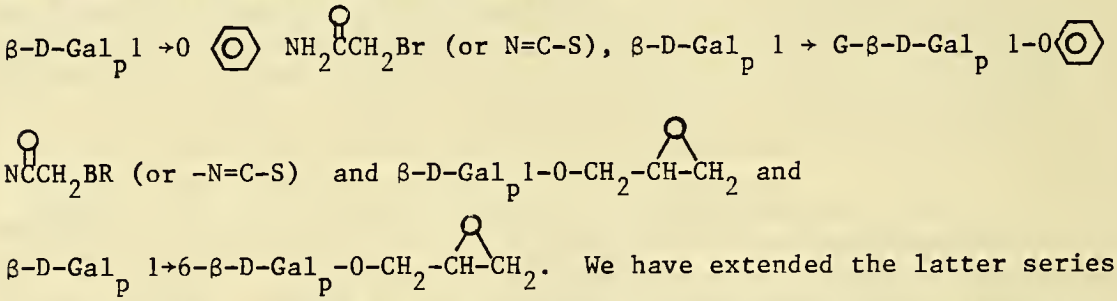
A. The study of H-bonding between ligand and protein and the arrangement of subsites.

B. The preparation of a series of affinity labels for the anti-galactan monoclonal antibodies and the preparation of saccharides for binding studies.

Recent Work

Project A: A series of synthetic β1,G galactosyl oligosaccharides were prepared having selected hydroxyl groups replaced by fluorine atoms. These ligands were then studied for their binding to monoclonal IgA J539. From the data it could be derived that the antibody has four subsites (one for each galactosyl residue of the antigen chain it binds) and that their affinity decreases from in the order A > B > C > D. When these sites are aligned as CABD in going from the Heavy (H) to light (C) chain across the face of the IgA combining area.

Project B: In the recent past we have prepared two kinds of affinity labels for antigalactan antibodies:



β-D-Gal$_p$1 →O ⟨⬡⟩ $NH_2\overset{O}{\overset{\|}{C}}CH_2Br$ (or N=C-S), β-D-Gal$_p$ 1 → G-β-D-Gal$_p$ 1-O⟨⬡⟩

$N\overset{O}{\overset{\|}{C}}CH_2BR$ (or -N=C-S) and β-D-Gal$_p$1-O-CH_2-$\overset{O}{\overset{/\backslash}{CH}}$-$CH_2$ and

β-D-Gal$_p$ 1→6-β-D-Gal$_p$-O-CH_2-$\overset{O}{\overset{/\backslash}{CH}}$-$CH_2$. We have extended the latter series of compounds, these being highly reactive ligand containing galactosyl residues.

SECTION ON MEDICINAL CHEMISTRY

BIOCHEMICAL-PHARMACOLOGICAL INVESTIGATION OF OPIOIDS AND STIMULANTS/DEPRESSANTS

Opioid-like compounds and those in the stimulatant/depressant classes are examined in a number of biochemical and pharmacological paradigms to discern their physical dependence potential and abuse liability, under the auspices of the Committee on Problems of Drug Dependence.

There were several compounds examined of practical and/or theoretical importance. Four compounds were submitted by the DEA, through NIDA, which might be called "street" or "designer" drugs. Two of them are extremely potent analogs of fentanyl, the cis and trans 3-methyl fentanyl (NIH 10456 & 10457). The more potent cis compound was found to be 1000 to 2000 times more potent than morphine in mouse antinociceptive assays (hot plate, PPQ, tail flick), and was 1000 times more potent than morphine in the single dose suppression assay in monkeys. Its affinity to opioid receptors in rat brain membranes and in the mouse vas deferens was in reasonable accord with the in vivo data. The somewhat less potent trans compound was 600 times more potent than morphine in suppressing abstinence in the single dose suppression study. These appear to be quite dangerous drugs for street sale. Two far less potent pethidine-like compound, NIH 10460 and 10461 also completely suppress abstinence in single dose suppression studies and were as potent as, or more potent than morphine in the various antinociceptive studies in rodents.

Agonists and Antagonists for the Phencyclidine Receptor.

Metaphit, the first electrophilic affinity ligand specific for phencyclidine receptors, has been found to be a very useful compound for the study of phencyclidine receptors in various brain areas. Other ligands for the PCP receptor, including affinity ligands, have been synthesized for biochemical and pharmacological investigation.

A number of compounds were synthesized based on 2-methyl-3,3-diphenyl-3-propanolamine (2-MDP), which had been found to have PCP-like activity in vivo. Our biochemical work indicated that 2-MDP's affinity to the PCP receptor was about half that of PCP itself. The newly synthesized compounds are being explored for their activity in vivo. New structural types of affinity ligands for the PCP receptor are being explored, as well as compounds which might act as PCP antagonists. [A. E. Jacobson, M. V. Mattson, K. C. Rice, R. A. Lessor, A. Thurkauf].

Characterization of Opioid Receptors Using Rigid Probes

A series of relatively rigid molecules, from the endoethenooripavine family of opioids known to bind to opioid receptors from rat brain cerebrum homogenates and neuroblastoma-glioma hydride cells, have been found to act as irreversible binding ligands for the delta and mu opioid receptors. New synthetic methods for preparation of a simpler opioid family were explored as the base for potential kappa opioid affinity ligands, to explore the nature and function of that receptor system in the CNS. [A. E. Jacobson, R. A. Lessor, K. C. Rice, W. A. Klee].

CHARACTERIZATION OF OPIATE RECEPTORS USING NONRIGID IRREVERSIBLE INHIBITORS.

Chemical and biochemical studies of nonrigid irreversible inhibitors have been continued in several areas in order to gain more insight into the structure and function of opiate receptor subpopulations. The delta receptor subpopulation from NG 108-15 cells has been purified to homogeneity and characterized as an Mr 58,000 glycoprotein. Anatomical distribution of kappa opiate receptors in rat and guinea pig brain slices has been determined. The rat pituitary has been shown to contain almost exclusively kappa opiate receptors. Morphine tolerance in rats has now been shown to produce upregulation of [^{3}H]DADL binding sites. Studies aimed at synthesis and identification of a kappa selective irreversible ligand are in progress and will be reported in due course (K. C. Rice, W. Klee, A. Jacobson, W. Simonds, B. DeCosta).

TOTAL SYNTHESIS OF OPIATES VIA DIHYDROTHEBAINONE AND DERIVATIVES.

As previously described, the NIH Opiate Total Synthesis renders either enantiomer of morphine, codeine and thebaine freely available in 25-30% overall yield from m-methoxyphenthylamine. Modification of the synthetic sequence has afforded direct access to N-cycloalkyl methyl derivatives of thebaine which in turn has provided more direct access to the narcotic antagonists naltrexone and nalmefene as well as nalbuphine. Synthetic studies in the unnatural opiate series have been continued to provide samples for antitussive testing, study of the mechanism of cough, and as potential ligands for the phencyclidine receptors. Bivalent agonist and antagonist ligands containing either natural and unnatural opiate moieties or 2 natural moieties have been synthesized. Further modifications aimed at increasing the versatility of the NIH route are in progress and will be reported in due course. (K. C. Rice, A. Newman).

PHARMACOLOGICAL PROBES OF BENZODIAZEPINE RECEPTORS.

"Peripheral" benzodiazepine receptors (PBR) were initially demonstrated in the kidney, but are now known to be present in both the periphery and the CNS. We have now designed and synthesized the first two irreversible ligands (AHN 086 and AHN 070) which are specific for these sites (to the exclusion of the central benzodiazepine receptors) as probes of the structure and function of PBR. Although these two drugs have diverse chemical structures, they exhibit similar apparent IC_{50} values of 1-2 nM against [^{3}H]Ro 5-4864 and [^{3}H]PK 11195 (both reversible ligands specific for PBR) on PBR from brain and kidney. Both AHN 070 and AHN 086 showed a concentration dependent irreversible reduction of Bmax against [^{3}H]Ro 5-4864. It seems likely that these drugs will be highly useful in our continuing study of the structure and function the central and of PBR. (K.C. Rice, A. Newman, H. Luddens, P. Skolnick).

A procedure for high performance fast affinity chromatography of antibenzodiazepine antibodies has been developed in collaboration with S. Paul (NIMH) and associates.

SYNTHESIS AND EVALUATION OF POTENTIAL CNS, ANTIINFLAMMATORY AND ANTICANCER DRUGS.

A portion of our studies of the mechanism of action of phencyclidine (PCP) have involved synthesis and biochemical study of alkylating and acylating ligands as probes of the structure and function of PCP receptors. METAPHIT, the 3-isothiocyanatophenyl derivative of PCP proved to be a highly functional irreversible ligand in this regard. The isomeric 4-isothiocyanato-piperidine derivative of PCP, FOURPHIT, was however ineffective, thus showing structural specificity in this series. The latter agent has now been found to irreversibly increase the apparent affinity of the calcium antagonist [^{3}H] nitrendipine, for binding sites from rat forebrain. PCP reversiblity produced such a dose related increase, however METAPHIT was much less potent than FOURFIT in producing the increase of affinity for [^{3}H]nitrendipene. These results, together with the observation that PCP protects against the irreversible increase produced by FOURFIT suggest this agent acts at the same site as PCP, and that it will be a useful tool for further biochemical studies of the nitrendipine binding site. (K. Rice, G. Bolger, P. Skolnick).

CHARACTERIZATION OF OPIATE RECEPTORS USING POSITION EMISSION TRANSAXIAL TOMOGRAPHIC (PETT) IMAGING

Our earlier synthetic and biochemical studies resulted in the identification of the fluorinated narcotic antagonist [^{18}F]cyclofoxy as a highly functional ligand for PETT imaging of opiate receptors in the living baboon brain. We have now tritiated this ligand to high specific activity and further characterized its binding by autoradiography in rat brain sections as being essentially identical with that of (-)-naloxone. Radiochemical synthesis of the [^{18}F]cyclofoxy has been refined to radiochemically pure material of high chemical purity. Cyclofoxy is a potent narcotic antagonist in the rat tail flick vs. morphine test for narcotic antagonist activity, and together these data suggest that the ligand will prove suitable for imaging opiate receptors in the brain of conscious humans. (K. C. Rice, S. Larson, M. Channing, A. Newman, A. Pert, N. Ostrowski, C. Pert).

COLCHICINOIDS:

3-Demethylthiocolchicine continues to look good as a broad spectrum antitumor agent. It is like colchicine and demecolcine not very active orally. 2,3-Didemethylcolchicine did not bind well to tubulin protein in vitro, but inhibited the carrageenin induced edema in rat pads considerably, suggesting that antiinflammatory action of colchicinoids may not be regulated through microtubules. Colchicides, lacking the 10-methoxy group in the corresponding colchicinoids, did not show good binding affinity to tubulin protein, suggesting that the 10-methoxy group in colchicine is involved in the tubulin-binding process. A ring-open colchicine prepared at the University of Auckland in New Zealand showed no affinity to tubulin protein, supporting the view that ring B of colchicine is important, and that atropoisomerism of the molecule may occur in solution. The data accumlated over many years will now allow a conformational analysis of the colchicine molecule by computer modeling. (A. Brossi, R. Dumont).

PERHYDROHISTRIONICOTOXINS:

Two C-9-butylated ketolactams prepared by synthesis afforded by reduction with LAH amino alcohols which could be separated by chromatography. They constitute isomers of the biologically active 2-desamylperhydrohistrionicotoxin and will be compared with the latter in frog sciatic nerve muscle preparations. As a byproduct the first geminal 9,9-dibutylated lactam was obtained serving as an intermediate to prepare trialkyl-substituted spiroaminoalcohols. (A. Brossi, W. Gessner).

ANTIMALARIALS:

The mode of action of the tissue schizonticide primaquine is not well understood. Blocking the primary amino group by acylation, or removing the amino group by metabolic conversion to a carboxylic acid, is accompanied with a complete loss of antimalarial activity. The optical isomers of primaquine have different toxicities with the (+)-isomer being considerably less toxic to mice, but having the same antimalarial effect. Photooxidation of N-acylated primaquine afforded o-quinones in high yield, now for the first time available by practical procedures. The Chinese antimalarial qinghaosu is not very soluble and requires large doses for treatment. Reduction of qinghaosu with sodium borohydride, etherification of the lactol with ethanol in the presence of acid and chromatograhpic separation of the two ethyl ethers afforded β-dihydroquinghaosuethyl ether named arteether. Arteether is about 4 times more potent than qinghaosu and will be clinically tested in an oily formulation. This program is being sponsored by SWG-CHEMAL of WHO. (A. Brossi, B. Venugopalan, W. Gessner).

MAMMALIAN ALKALOIDS:

Alkaloids detected in alcoholics, phenylketonurics, and L-dopa treated Parkinsonian patients are called Mammalian alkaloids. Their function is not well understood. We now have for the first time prepared optical isomers of salsoline-1-carboxylic acid and salsolinol-1-carboxylic acid. Their configuration was determined by an X-ray analysis of (+)-1-carbomethoxy-salsoline hydrobromide.

The level of morphine, detected in brains of experimental animals, was considerably enhanced by administration of natural (-)-thebaine and natural (+)-salutaridine, both precursors of morphine in the poppy plant. Levels of (-)-codeine, the immediate precursor in the poppy biosynthesis of morphine, were particularly high. The suggestion that morphine may not only be a plant alkaloid but an endogenous mammalian alkaloid as well, is greatly advanced with these findings. (A. Brossi, K. C. Rice, R. Dumont, C. Schoenberger).

1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE (MPTP):

A study of deuterated analogs of MPTP has shown that oxidation of MPTP at the 6-position is a major rate-determining step in its biotransformation by MAO B. The neurotoxin MPTP is a pseudosubstrate for the copper oxidase ceruloplasmin and both, $MPDP^{+}$ and MPP^{+}, are produced. Incubation of MPTP with horseradish peroxidase only provided MPTP-N-oxide. Minor secondary metabolites represented of pyridone structure were prepared. The extreme specificity of MPTP towards MAO B was further supported with the inactivity found in this assay for 3- and 5-methylated analogs and for an N-phenylethyl substituted analog. (A. Brossi, W. Gessner).

PHYSOSTIGMINE AND ANALOGS

Improved synthesis of natural (-)- and unnatural (+)-physostigmin has been achieved. Critical step is the fragmentation of methylbenzylureas prepared from (±)-N1-noreseroline-O-methyl ether in refluxing butanol. The generality of this reaction was successfully studied with other examples, including : salsolidine, mecamylamine, tetrahydroharmine, 1-carbomethoxysalsoline and N-vanillylamphetamine. Modification of the carbamate moiety in natural (-)-physostigmine afforded in (-)-N-methylphysostigmine a congener of much higher potency, whereas unnatural (+)-physostigmine was a much less potent inhibitor of acetylcholinesterase. An X-ray analysis of rubreserine obtained by oxidation of eseroline was carried out. (A. Brossi, B. Schönenberger, C. Schoenberger).

SECTION ON METABOLITES

The Nicotinic Receptor Site and Tricyclic Antidepressants:

The actions of two clinically important dibenzocycloheptane antidepressant drugs, amitriptyline and nortriptyline, were studied on ionic channels of nicotinic acetylcholine (AcChR) receptors at the neuromuscular junction of frog skeletal muscle. Amitriptyline (5-10 μM) and nortriptyline (1-2μM), like imipramine (5-10μM), did not react with the nicotinic AcChR receptor but caused a voltage- and time-dependent decrease in the peak amplitude of the endplate current (epc). The time constant of epc decay, however, retained its voltage sensitivity. The voltage and time-dependent effect of amitriptyline was nonlinear with regard to the current/voltage (I/V) relationship. Nortriptyline also had a more pronounced voltage- and time-dependent effect evidenced by a hysteresis loop in the I/V relationship of the epc due to the drug's greater potency at more negative potentials. The nonlinearity and hysteresis loop in the I/V relationship of the epc was eliminatd bythe use of 50-msec stepwise changes of the membrane potential. The nonlinearity and hysteresis were due to a time-dependent phenomenon and did not involve previous AcCho receptor activation. The rate constant of the voltage- and time-dependent decrease in epc amplitude was sensitive to the membrane electric field and varied linearly with the membrane potential. Iontophoretically elicited epcs were much more depressed by both drugs than were spontaneous miniature epcs. There was no effect on the time constant of miniature epc decay, single-channel lifetime, or conductance. Thus, as was pointed out in our histrionicotoxin studies the primary site of action of these agents presumably is the activated but nonconducting species of the ionic channel of the nicotinic AcCho receptor. These agents, particularly nortriptyline, point to several different binding sites of the ionic channel and are suitable tools for the separation of the effects on peak current amplitude from its time constant of decay (G. Schofield, B. Witkop, J. E. Warnick and E. X. Albuquerque).

Molecular Models of the Acetylcholine Receptor:

A process of elimination helps to refine current computer-assisted molecular models of the n-AcChR as published by Robert M. Stroud, S. Numa or Homer R. Guy. A somewhat different model has been suggested by E. M. Kosower (Tel Aviv) whose findings were presented in May at an international conference on the island of Santorini (Greece) and are in the process of publication in consultation with B. Witkop.

Modifications of Cocaine for the Preparation of Novel Nicotinic Agonist and for the Reactivation of Receptors Poisoned by Organophosphorus Agents:

Cocaine has been used for ring enlargements to arrive at nicotinic agonists of the anatoxin-a type, for the preparation of unsaturated ketones of the ferruginine type and more recently for the elaboration of ketonic derivatives. 2-Tropinone oxime reactivates the acetylcholine receptor, inactivated by nerve gas, such as Soman (R. Moriarty, University of Illinois at Chicago). This research topic, suggested three years ago to Professor Robert Moriarty, has become a major and now independent research project supported by Funds from the U. S. Army for five years.

The Search for New Membrane-Active Substances: Synthesis of Tropan-3-ols with Alkyl, Alkenyl and Alkenynyl Groups at the Bridgehead:

These synthetic studies were prompted by the unique structures and properties of the naturally occurring histrioncotoxins, valuable tools for probing ion flux in nicotinic receptor sites. A contract arrangement between NIDDK and Professor Gabor Fodor, University of West Virginia, Morgantown, West Virginia, produced several new candidate compounds expanding on derivatives of the ant-toxin Adaline previously synthesized by Gössinger and Witkop.

Modeling Studies on AchR Agonists:

The specific structures are: (+)anatoxin-a (unprotonated and protonated); methyl (protonated) and Dimethyl anatoxin-a, nor-ferruginine (unprotonated and protonated); Ferruginine (protonated), Ferruginine methiodide and isoanatoxin-a (unprotonated and protonated).

The compounds were first modeled by means of the Merck Molecular Modeling (MMMS). The structures were then fully energy-geometry optimized using Allinger's Molecular Mechanics (MM2) Program. Prior to modeling, the latter program was altered to handle charges. Structural parameters for ammonium slats were generated from X-ray diffraction data on methyl, polymethyl, penta- and hexacyclic ammonium slats. Charge parameters were derived from 6-31G* ab initio calculations on several of the salts. Results obtained from MM2 using these parameters agree very well with the ab initio and MNDO calculations for charge densities and relative energies. Steric energies; C=C-C=- dihedral angles for the s-trans and s-cis isomers and the non-bonded distances between the carbonyl oxygen at its Vander-Waals extension and the nitrogen were calculated and correlated. For the unprotonated and protonated anatoxin-A, only the boat conformations were observed for the seven-membered ring, and two twisted conformations for the five-membered ring. The difference in energy between the latter two is estimated to be about 1.0 kcal. For the in protonated and protonated anatoxin-a, the s-trans conformer is lower in energy than the s-cis by about 0.5 kcal. For the protonated anatoxin-a, the difference between the s-trans and the s-cis siomers is larger by about 8.0 kcal. This calculation was checked with MNDO with the found results. We are not yet sure what meaning, if any, should be ascribed to the dihedral angles for the s-trans and s-cis. Nonetheless, the measured non-bonded distances between the nitrogen and Vander Waals radius of the carbonyl oxygen indicate that the s-cis siomers have the proper Beer's and Reich disance, whereas for the s-trans isomers this distance is too short. The lowest energy conformer for protonated methyl and dimethyl anatoxin-a is the chair (PMANA12A and DMANAØ1). However, in both cases the nitrogen to carbonyl oxygen distance is very short (@ 4.1A°). Again, only the s-cis conformers attain the Beers and Reich distance. We took the s-trans-chair conformer (PMANA12A) and rotated the C_2-C_3 single bond in $c=c_2-c_3=0$ from 180 to 0°. The energy of the s-cis conformer was about 9 kcal higher and the chair was still present though there is no calculatd barrier between the two rotamers. For the dimethyl anatoxin-a, the s-cis isomer exits only in the boat. We have performed a calculation between -066° the boat prevailed (Energies = 31→29 kcal) while at 96° the chair conformer formed (Energy = 17.4 kcal) and achieved its lowest energy at 156° (Energy - 12.1 kcal). The unprotonated and protonated

nor-ferruginines, ferruginines and ferruginine methiodides show similar results, except that the six-membered ring is relatively flat. Again the s-trans isomers are of lower energy than the s-cis by about 8 kcal, and the Beers and reich distance can only be reached by the S-cis. The isoanatoxin series shows the chair to be of lower energy than the twist boat conformers for the unprotonated case, and the s-trans .5 kcal lower in energy than the s-cis. The Beers and Reich distance is obtained for the s-cis and it doesn't matter if the second six membered ring is chair or twist boat. For the protonated isoanatoxin-a only the chair is obtained, s-trans below s-cis by 8 kcal, and the Beers and Reich distance can only be obtained by the s-cis. These results in general predict that if the distance criterion is most important, then all of these should bind to the receptor.

These model studies, now done independently by Dr. Tamara Gund, New Jersey Institute of Technology, Newark, N. J., are now important enough to be supported by the US Army with a grant approaching $0.5 million.

Chirality in Bioactive Agents: New Discovereis in the Family of Physostigmine Alkaloids:

Enantioselectivity in "hybrid" (racemic) drugs has been a subject of concern to leaders in the field of medicinal chemistry, such as E. J. Ariëns and A. Brossi. The evaluation of therapeutic and toxicological data from racemic products leads to highly misleading data. As a corollary to the symposium on "Membrane Biochemistry and Organophosphorus Agents", organized by B. W. Agranoff, A. G. Karczmar and B. Witkop at West Palm Beach, Florida, on November 11-13, 1984, the preparation and resolution of the enantiomers of physostigmone and eseroline in the Section on Medicinal Chemistry was carried out by A. Brossi and clearly defined the properties of the unnatural (+)-physostigmine as a much weaker inhibitor of choline-esterase than the natural (-)-physostigmine. Likewise, (+)-eseroline still binds to opiate receptors but is not a potent narcotic analgesic like (-)-eseroline.

Optical Antipodes of Agonists (+) and (-)-Anatoxin a:

The synthesis of antipodes of anatoxin a, originally projected to e done within the Laboratory of Chemistry, by Drs. A. Brossi and Dr. Gessner, has now been accomplished at University of California, Berkeley by arrangement with Professor Henry Rapoport, starting with D- and L-glutamic acid, respectively. These antipodes are now under electrophysiological investigation in the Laboratory of Professor Edson X. Albuquerque, whose most important finding is that anatoxin desensitizes the acetylcholine receptor at a speed considerably slower than acetylcholine, the natural agonist.

Neurochemical Aspects of Alzheimer's Disease:

A collaborative program with Dr. Stanley Rapport, Nat. Inst. of Aging, has been initiated to screen agonists for candidates in connection with Alzheimer's disease, the largest single cause of loss of memory function in the elderly population of the Western world, in most cases a rather specific disorder of cholinergic neurons. It should be possible to develop effective treatments for the symptoms of this disease. It is only recently that Alzheimer's disease has come to the attention of the majority of the public and scientific communities. One reason for this sudden rise to fame has been

the recognition that at least half of the elderly patients said to be suffering from 'senility'; 'organic brain syndrome' or 'hardening of the arteries' actually have in the brain the pathologic signs of Alzheimer's disease, without any other significant brain disease. The direct examination of brain tissue to reveal the presence of these pathological features, the neurofibrillary tangle and the neuritic plaque, remains the only way to definitively diagnose this disease. The best estimates of the size of this patient group suggest that there are about 1.5 million such individuals in the USA alone. Hence the recent attention given to Alzheimer's disease seems both justified and long overdue. Attempts to enhance central cholinergic transmission must be selective: non-selective enhancement of acetylcholine release could clearly result in enhanced transmission in pyramidal and extrapyramidal motor systems, probably producing undesirable side-effects. What is required is the ability to enhance cholinergic transmission in the nucleus basalis cortical system without disruption of normal transmission at other cholinergic synapses. One high priority area for reearch might be to search for agents which are muscarinic or nicotinic agonists selective for receptors in cortical and hoppocampal regions. Another approach might be to selectively enhance the sensitivity of muscarinic or nicotinic acetylcholine receptors on cortical and hippocampal neurons. Other possibilities include a search for receptors activating cholinergic neurons of the nucleus basalis, to attempt to drive these cells to release more acetylcholine into the deficient regions. Methods to enhance acetylcholine synthesis at terminals within the cortex and hippocampus may also exist; it would appear that only a small fraction of the choline acetyltransferase in synaptic terminals is active in acetylcholine synthesis, although the reasons for this are far from clear. Another possible therapeutic avenue is the only one that has so far produced reproducible improvements in patients in double blind studies. Patients have been treated with oral physostigmine, a naturally occurring cholinesterase inhibitor (usually in combination with lecithin) or synthetic cholinesterase inhibitors, such as prostigmine or pyridostigmine. The new pure enantiomers of eserine and eseroline will now be tested in this program, as well as in the Laboratory of Dr. Kenneth J. Kellar, Georgetown University, in which nicotinic acetylcholine binding sites have been shown to be involved in Alzheimer's Disease.

Physostigmine and Pyridostigmine as Weak Nicotinic Agonists:

Completely unexpected effects, found for these inhibitors in the Laboratory of Professor Edson X. Albuquerque, have now to be taken into consideration.

When the actions of pyridostigmine (Pyr), an anticholinesterase agent, were studied on the acetylcholine (ACh) receptor-ion channel complex and on the electrically excitable membrane of the frog cutaneous complex and on the electrically excitable membrane of the frog cutaneous pectoris and sartorius muscles and the chronically denervated soleus muscle of the rat, Pyr at concentrations of 0.2-0.4 mM potentiated the indirect evoked muscle twitch and at concentrations $\geq$ 0.8 mM depressed the indirect twitch with an IC_{50} of about 2 mM. Twitch depression produced by Pyr was reversed slowly, and after a 60-min wash only 59% of the control muscle twitch had returned. Pyr did not affect either the membrane potential or the muscle action potential. Pyr had several effects at the neuromuscular junction of the frog and rat. It decreased the peak amplitude of the end-plate current (EPC) in a voltage- and

conentration-dependent manner. In contrast to diisopropylfluorophosphate, which depresses the EPC amplitude and induces a double exponential decay of the EPC and miniature end-plate current (MEPC), Pyr produced a marked prolongation of the time constants of EPC and MEPC decay while maintaining a single exponential decay. The decrease caused by Pyr of indirect twitch tension, EPC amplitude, and ACh sensitivity indicates mechanisms which limit the number and/or properties of conducting channels. The drug decreased channel conductance and prolonged channel lifetime as revealed by Fourier analysis of ACh-induced end-plate currently fluctuations. An altered form of the conducting species induced by Pyr appears to be responsible for either the apparent agonist-induced depolarization or its ability to increase the affinity of ACh for its recognition site. Pyr was also found to inhibit the binding of ACh and α-bungarotoxin to receptor-rich membrane from the electric organi of Torpedo nobiliana, and to have a higher affinity for the receptor than for the ion channel binding sites. These actions are distinct from acetylcholinesterase inhibition caused by the agent. Strong evidence suggests that the direct influences of the agent on neuromuscular transmission involve at least three distinct, although possibly interacting, mechanisms: (a) a weak agonist action, (b) the formation of desensitized receptor-complex intermediates, and (c) the alteration of the conductance properties of active channels.

As this contribution from the Laboratory of E. X. Albuquerque reveals, both physostigmine and pyridostigmine act as weak agonists with the acetylcholine receptor-ionic channel complex and are capable of inducing desensitization. This phenomenon of desensitization may probably be responsible for numerous cases of death observed after administration of high doses of physostigmine or prostigmine to patients suffering from myasthenia gravis.

The U. S. Army has spent several hundred million dollars for the preparation of pyridostigmine on a level large enough to provide protection for the Armed Forces. A better and more efficient protection without side effects might be provided by (+)-physostigmine the unnatural synthetic enantiomer of the natural occurring alkaloid eserine ((-)-physostigmine), provided the problem of logistics can be overcome. One major hurdle, efficient resolution, has been taken by A. Brossi's extremely efficient and simple thermolysis of diastereoisomeric urea derivatives.

Birth and Rebirth of Leading Ideas:

A lecture under this title wad delivered on the invitation of the Medical Faculty of the University of Frankfurt. Progress in science depends on progress in methods. More than 2000 Protein sequences were elucidatd with the help of two chemical cleavage methods, the Edman degradation and the cyanogen bromide cleavage. What is less well known is the logical and historical connection that exists between the timing and origin of the cyanogen bromide cleavage and the development of the first gel-based radioimmunoassay techniques by Jerker Porath and Rolf Axen. As Professor Porath described this sequence of events in his own words:

> "Rolf Axen joined Bernhard Witkop at Bethesda to learn new techniques for selective cleavage of protein. Upon his return to the Institute of Biochemistry at Uppsala, he suggested a new approach to our

project, viz., the coupling of substances containing primary amino groups to cyanamide-Sephadex. The idea was suggested as a research project to Sverker Ernback, one of our first-year research students.

"The cyanamide-Sephadex was prepared by treating amino-Sephadex with cyanogen halide. To my surprise, the yield of coupled amino acid exceeded 100 percent! I urged Ernback to make blind experiments using unsubstituted Sephadex. Indeed, Sephadex after cyanogen halide treatment was found to immobilize protein in excellent yields!

"Cyanogen bromide coupling replaced the isothiocyanate procedure for the synthesis of immobilized antigens and antibodies to be used in radioimmunoassays. It is still the most commonly used method for preparing gel- and paper-based immunodiagnostics (RIA, RAST, PRIST, etc.).

"Our discovery of the cyanogen halide coupling method was followed by hectic work in several directions. Enzymes and enzyme inhibitors were immobilized onto a variety of hydroxylic supports. Not much later, our work on activation of agarose for enzyme immobilization was described, and this work initiated an almost explosive development in (bio-)affinity chromatography.

"We interpreted the activation to involve the formation of cyanate followed by its rapid conversion to imino carbonate. The final coupling products were thought to be gels containing mixtures of imino carbonic acid esters, carbonic acid eters, and carbamate substituents, and, somewhat later, isourea linkages were also considered. Evidence for this interpretation was obtained from IR-spectra including also some model compounds."

M. Wilchek, like Axen a Visiting Fellow in the Laboratory of Chemistry, determined the active species in cyanogen bromide-activated polysaccharides.

"The complicated scheme of reactions is now fairly well understood, thanks to Meir Wilchek and others. The nucleophilic displacement of the ligands with ammonia is particularly interesting: original amino groups are converted into guanidino groups. By using the cyanogen bromide activated support as an organic reagent, Wilchek converted insulin into 'superinsulin'.

"Our original suggestions have been essentially confirmed, but the recent work has shed light on some important limitations. In improved form, the cyanogen bromide coupling is still the preferred method for the preparation of most biospecific adsorbents used in (bio)affinity chromatography, and agarose is by far the most commonly employed support." [Cf. Current Contents, This week's Citation Classic, May 28, 1984, Number 22, p. 21].

Consulting Activities:

Close to 100 kg of human insulin are now being produced at Ely Lilly per annum by liberating it from a bacterial chimeric protein made by genetic engineering at the ethionyl peptide link by the use of cyanogen bromide.

Another application of the selective cyanogen bromide cleavage, in consultation with Sidney Udenfriend, Roche Institute, will be on the mμ-type opiate receptor, 65000 daltons, isolated from bovine caudate-putamen, which has a blocked amino terminus and cannot be sequenced by the micro Edman procedure.

The Reaction Site of A Noncompetitive Antagonist in the δ-subunit of the Nicotinic Receptor:

Two valuable tools from the LC were used by F. Hucho, Berlin, to localize the binding site for the non-competitive antagonist Histrionicotoxin at position 262 in the δ-subunit of the acetylcholine receptor complex. The position of this crucial amino acid was ascertained by differential cleavage with cyanogen bromide, a procedure discussed with F. Hucho whose manuscript was prepared, modified, edited and accepted for FEBS Letters.

Selective Modifications of Cyclosporin A:

The cyclic endecapeptide cyclosporin A from trichoderma polysporum possesses one unsubstituted methylene group in its glycine residue which forms a carbanion in tetrahydrofuran with excess buty-lithium (Seebach, 1985). Alkylation in this position with suitable methylmercaptoethyl substituents should lead to methionylcyclosporin analogs. Selective cleavage with cyanogen bromide opens a way to seco-cyclosporins in which addition or substraction of one or more amino acid resiidues and reclosure opens up a route to lower or higher homologs of cyclosporin whose clinical importance is rising day by day (joint project with Dr. Kurt Freter, Boehringer, Danbury, Connecticut).

Thirty Years of Stewardship at the Laboratory of Chemistry 1957-1987:

In 1987 B. Witkop will end his role as Chief of the Laboratory as he completes his 70th year of life. This is his last Annual Report.

Synthesis and Biological Activityof Uridine-Substituted Analogs of 2-5A

Earlier studies have demonstrated that the second (from the 5'-terminus) nucleotide residue of 2-5A [5'-O-triphosphoryladenylyl(2'→5')adenylyl(2'→5') adenosine] could be replaced by either inosine or 7-deazaadenosine without adversely affecting ability to bind to or activate ribonuclease L of mouse cells. To determine how drastic a change in heterocyclic base this second nucleotide unit of 2-5A might accomodate, the second adenosine residue was replaced by uridine through a synthesis which involved a modification of the lead ion-catalyzed ligation reaction. The product, ppp5'A2'p5'U2'p5'A, was 100-1000 x less active than 2-5A itself as an activator of RNase L as determined by inhibition of translation and ability to cause degradation of a synthetic RNA. This loss of activity was associated with a concomitant loss of RNase L binding ability as ascertained by the ability of p5'A2'p5'U2'p5'A to antagonize the translational inhibitory action of 2-5A. Thus, some structural element of the adenosine ring other than the purine N1/6 amino group and purine N7 may contribute to the interaction of 2-5A with its target enzyme, RNase L.

Synthesis and Biological Activity of a Guanosine and 7-Deazaadenosine Substituted Analog of 2-5A: p5'G2'p5'(c^{7}A)2'p5'(c^{7}A)

A guanosine and 7-deazaadenosine-substituted analog of 2-5A has been prepared: viz, p5'G2'p5'(c^{7}A)2'p5'(c^{7}A). Its synthesis involved the lead ion-catalyzed condensation of the dimer A3'p5'G with the 5'-phosphoro-imidazolidate of the dinucleotide p5'(c^{7}A)2'p5'(c^{7}A) to yield the tetramer A3'p5'G2'p5'(c^{7}A)2'p5'(c^{7}A). This tetranucleotide was digested with ribonuclease P$_1$ to cleave the single 3',5'-phosphodiester linkage to give, after purification, p5'G2'p5'(c^{7}A)2'p5'(c^{7}A). When evaluated for its ability to bind to the 2-5A-dependent endonuclease of mouse L cells, it was found to have an activity least a 10,000 times less than 2-5A itself or the parent trinucleotide 5'-monophosphates, p5'A2'p5'A2'p5'A or P5'(c^{7}A)2'p5'(c^{7}A)2'p5'-(c^{7}A). The results verify the importance of purine N1/N6 amino residue in determining binding to RNase L.

2',5'-Phosphodiesterase Activity Depends Upon the Presence of a 3'-Hydroxyl Moiety in the Penultimate Position of the Oligonucleotide Substrate

A series of 3'-deoxyadenosine (3'dA, cordycepin)-substitued analogs of 2-5A core 5'-monophosphate (p5'A2'p5'A2'p5'A) were examined for thier sensitivity toward degradation by the 2'-phosphodiesterase activity of cytoplasmic extracts of mouse L cells. The analogs, p5'(3'dA)2'p5'A2'p5'A, p5'(3'dA)2'p5'A2'p5'(3'dA) and p5'A2'p5'A2'p5'(3'dA) were degraded only somewhat less slowly thatn p5'A2'p5'A2'p5'A itself. On the other hadn, under the assay conditions examined p5'A2'p5'(3'dA)2'p5'A, like p5'(3'dA)-2'p5'(3'dA)2'5'(3'dA), was completely resistant to degradation. The data imply that sensitivity to the 2',5'-phosphodiesterase activity of mouse L cells, requires the presence of 3-hydroxyl moiety in the penultimate nucleotide. (Alster, Brozda, Kitade, Wong, Torrence).

Respective Role of Each of the Purine N7 Nitrogens of 2-5A in Biding to and Activation of the RNase L of Mouse Cells

Through a combination of chemical and enzymatic approaches a series of sequence-specific tubercidin-substituted 2-5A analogs were generated. In addition to the previously developed methodology of Imai and Torrence (1985), a new approach to synthesis of 2',5'-linked oligonucleotides utilized adenosine in 3',5' linkage to the targeted 5'-terminus of the desired product. For instance, A3'p5'A could be condensed under conditions of lead ion catalysis with tubercidin 5'-phosphate to give A3'p5'A2'p5'(c^{7}A). Treatment with the 3',5'-specific nuclease P$_1$ led to P5'A2'p5'(c^{7}A). The combined use of the above procedures led to the synthesis of p5'(c^{7}A)-2'p5'A2'p5'A, p5'A2'p5'(c^{7}A)2'p5'A, P5'A2'p5'A2'p5'(c^{7}A) and p5'A2'-p5'(c^{7}A)2'p5'(c^{7}A) which were converted to their corresponding 5'-triphosphates by the usual methods.

Evaluation of these analogs for their ability to bind to and activate the 2-5A-dependent endonuclease (RNase L) of mouse L cells showed that reltively little change occurred in the ability of the four tubercidin analogs to bind to RNase L. However, whenever the first and/or third adenosine nucleotide units were replaced by tubercidin, a dramatic decrease in ability to activate RNase L occurred. Only the second (from the

5'-terminus) adenosine residue could be replaced by tubercidin without any effect on RNase L activation ability.

Role of Each of the 3'-Hydroxyl Groups of 2-5A in Determining Biological Activity

Replacement of the 3'-hydroxyl moiety of adenosine wtih hydrogen gives cordycepin or 3'-deoxyadenosine. A cordycepin analogs of 2-5A, ppp5'(3'dA)2'p5'(3'dA)2'p5'(3'dA), could bind to the RNase L of mouse cells, albeit somewhat less effectively than 2-5A, but was incapable of activating the enzyme unless extremely high concentrations of analog were used. In the current effort, we have replaced sequentially most of the adenosines of 2-5A by cordycepin, viz., pp5'(3'dA)2'p5'A2'p5'A, ppp5'A2'p5'(3'dA)2'p5'A, ppp5'A2'p5'A2'p5'(3'dA) and evaluatd their ability to bind to and activate RNase L. We have found that only the 3'-hdroxyl moeity of the second adenosine residue of 2-5A is necessary for activation of RNase L. This is a most exciting discovery for it presents the opportunity for modification of at least one phosphodiester linkage to a triester to increase the possiblity of cellular penetration. In addition, now a replacement for the second 3'-hydroxyl group can be sought (e.g., F, N_3) to allow further phosphodiester modification.

Reactions and Immunochemistry of Carbohydrates

This laboratory continues the study of the interactions of antigens with (monoclonal) antibodies on the molecular level. The approach is two-fold.

A. The study of H. bonding between ligand and protein and the arrangement of subsites.

B. The preparation of a series of affinity labels for the anti-galactan monoclonal antibodies and the preparation of saccharides for binding studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 19001-14 LC
Formerly:
Z01 AM 19001-13 LC

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Reactions and Immunochemistry of Carbohydrates

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Cornelis P. J. Glaudemans	Chief	Section on Carbohydrates	NIDDK LC
Others:	A. K. Bhattacharjee	Expert		NIDDK LC
	G. L. Jung	Visiting Fellow		NIDDK LC
	P. Kovac	Visiting Scientist		NIDDK LC
	E. M. Nashed	Visiting Fellow		NIDDK LC

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Chemistry

SECTION
Section on Carbohydrates

INSTITUTE AND LOCATION
NIH, NIDDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5	5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This laboratory continues the study of the interaction of antigens with (monoclonal) antibodies on the molecular level. The approach is two-fold.

A. The study of H-bonding between ligand and protein and the arrangement of subsites.

B. The preparation of a series of affinity labels for the anti-galactan monoclonal antibodies and the preparation of saccharides for binding studies.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 19002-13 LC
Formerly:
Z01 AM 18005-12LBM

PERIOD COVERED
October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 charactars or less. Title must fit on one line between the borders.)*
B. Cell Proliferation: Mechanism of Triggering and Regulating Activation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Milton Kern	Research Chemist	NIDDK-LC
OTHERS:	Sibghat Ullah	Visiting Fellow	NIDDK-LC

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Chemistry

SECTION
Section on Carbohydrates

INSTITUTE AND LOCATION
NIH, NIDDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

To study the relatively late intracellular signals involved in the proliferative response of B lymphocytes to antibodies specific for surface membrane immunoglobulins, extracts from antibody activated cells were mixed with Xenopus laevis splenic nuclei and the incorporation of thymidine 5'-triphosphate into DNA assessed. The slight incorporation observed with either nuclei or extract alone was markedly enhanced upon mixing the two entities when the extract was derived from cells cultured with but not without anti-receptor antibody. The appearance of active extract correlated well with the culture requirements necessary for the induction of B lymphocyte proliferation and, as revealed by time course studies, the active component arises relatively late in the activation process. Moreover, the appearance of active extract is independent of DNA synthesis but is dependent on protein synthesis as judged from studies with metabolic inhibitors. Appropriate homogenizaton of activated cells yielded nuclei and cytoplasm with 85 percent of the activity confined to nuclei. In addition, purified active extracts exhibited DNA binding although the active component was readily distinguisable from polymerase α by chromatographic techniques. It is tentatively concluded that the active component represents either some replication protein other than polymerase or some earlier signal necessary to induce the formation or utilization or replicating proteins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 19200-36LC
Formerly Z01AM19200-35LC

PERIOD COVERED
October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biological-Pharmacological Investigation of Opioids and Stimulants/Depressants

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigetor.) (Name, title, laboratory, and institute affiliation)*

P.I.	A. E. Jacobson	Research Chemist	NIADDK-LC
OTHERS:	M. Mattson	Technician	NIADDK-LC

COOPERATING UNITS *(if any)*
Univ. of Michigan Med. School, and the Medical College of Virginia, Johns Hopkins Univ. Med. School, Univ. of Chicago Med. School and the Committee on Problems of Drug Dependence,Inc.

LAB/BRANCH
Laboratory of Chemistry

SECTION
Medicinal Chemistry

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.7	.2	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Opioid-like compounds and those in the stimulant/depressant classes are examined in a number of biochemical and pharmacological paradigms to discern their physical dependence potential and abuse liability, under the auspices of the Committee on Problems of Drug Dependence.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 19202-13LC
Formerly
~~Z01 AM19202-12 LC~~

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Synthesis and Evaluation of Potential CNS, Antiinflammatory and Anticancer Agents

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	K. C. Rice	Research Chemist	NIDDK-LC
	T. R. Burke, Jr.	Senior Staff Fellow	NIDDK-LC

COOPERATING UNITS *(if any)*

NIDDK-LBC (P. Skolnick, G. Bolger), NCI-LB (C. Klee, D. Newton)

LAB/BRANCH

Laboratory of Chemistry

SECTION

Medicinal Chemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Phencyclidine is known to allosterically increase the apparent affinity of the dihydropyridine (tritiated nitrendipine) calcium antagonist binding site in a lysed synaptosomal membrane preparation of rat forebrain. Treatment of a similar preparation of mouse forebrain with 4-isothiocyanato-1-(1-phenylcyclohexyl) piperidine (FOURPHIT), an acylating phencyclidine derivative, resulted in a concentration dependent (.1-10μM), irreversible, increase in the apparent affinity of tritiated nitrendipine in contrast to the effects of phencyclidine which were reversible. The FOURPHIT isomer, 1-[1-(3-isothiocyanatophenyl) cyclohexyl]piperidine (METAPHIT), (10 μM) also irreversibly increased the apparent affinity of tritiated nitrendipine, but was much less efficacious than FOURPHIT. Phencyclidine blocked the irreversible increase in the apparent affinity of tritiated nitrendipine produced by FOURPHIT. The interactions of multivalent cations and the calcium antagonist diltiazem with the tritiated nitrendipine binding site were altered following treatment of membranes with FOURPHIT. These studies suggest that FOURPHIT irreversibly interacts with the same sites as PCP to alter the binding of nitrendipine and thus may be a useful tool with which to further probe both the behavioral and biochemical interactions between phencyclidine and the dihydropyridine calcium antagonist binding site. METAPHIT was previously reported to acylate the classical PCP receptor believed to mediate many of the behavioral effects of PCP. FOURPHIT, however failed to acylate this site under the same conditions, indicating structural specificity for this interaction.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 19216-10LC
Formerly
Z01 AM19216-09LC

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure-Activity Relationships of Colchinoids Based on Tubulin Binding

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Arnold Brossi	Visiting Scientist	NIDDK-LC
Others:	Peter Kerekes	Visiting Scientist	NIDDK-LC
	Raymond Dumont	Visiting Fellow	NIDDK-LC

COOPERATING UNITS *(if any)*

C. Chignell, Res. Tri. Park, N.C.; M. Suffness/F. Quinn, NCI/NIH; M. Banwell, University of Auckland, New Zealand; J. Wolff, NIDDK, NIH; P. Sharma, School of Pharmacy, U of Kansas, Lawrence; Pierre Potier, Gif,CNRS, France; M.Ravid, Dept. of Medicine, Meir Gen. Hosp., Israel; Roussel-Uclaf Co., Paris, France.

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Laboratory of Chemistry

SECTION

Section on Medicinal Chemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Novel and known analogs of thiocolchicine were evaluated in vitro in a tubulin binding assay and in vivo in mice for acute toxicity and in the P388 lymphocytic leukemia assay. Selected compounds also were investigated in the carrageenin-induced footpad edema in rate for anti-inflammatory effects.
3-Demethylthiocolchicine (NSC 361792) showed broad spectrum antitumor activity, but was orally not active. Cornigerine and 2,3-didemethylcolchicine, but not their thiomethyl analogs showed potent antiinflammatory effects. A series of analogs of colchicide showed low tubulin binding affinity. An open-ring B analog of colchicine showed no tubulin binding affinity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 19226-08LC
Formerly
Z01 AM 19226-07LC

PERIOD COVERED: October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Pharmacological Probes of the Benzodiazepine Receptor

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	K. C. Rice	Research Chemist	NIDDK-LC
OTHERS:	A. Hauck-Newman	Guest Worker	NIDDK-LC

COOPERATING UNITS *(if any)*: NIMH-CP (S. Paul, R. Weber, M. Goldman), NIADDK-LBC (P. Skolnick, H. Luddens)

LAB/BRANCH: Laboratory of Chemistry

SECTION: Medicinal Chemistry

INSTITUTE AND LOCATION: NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A procedure has been developed for preparation of an affinity column suitable for rapid (<0.5h) purification of rabbit antibenzodiazepine antibodies. The antibodies can be obtained from the column directly suitable for iodoimmunoassay simply by neutralizing the appropriate fraction of column effluent. The first irreversible ligands specific for kidney and brain "peripheral" benzodiazepine receptors (to the exclusion of the "central" receptors) have been synthesized and identified biochemically. These compounds are isothiocyanate derivatives of Ro 54864 and PK 11195 and promise to be of enormous value in elucidation of the structure and physiologic function of the peripheral benzodiazepine receptors. Investigations along these lines are now in progress and will be reported in due course.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 19233-07LC
Formerly
Z01 AM 19233-06LC

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Total Synthesis of Opioids via Dihydrothebainone and Derivatives

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: K. C. Rice Research Chemist NIDDK-LC
OTHER: A. Hauck-Newman Guest Worker NIDDK-LC

COOPERATING UNITS *(if any)*

Research Triangle Institute, (F. I. Carroll); University of Minnesota (P.S. Portoghese)

LAB/BRANCH

Laboratory of Chemistry

SECTION

Medicinal Chemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0	.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The NIH Opiate Total Synthesis has been refined and its versatility extended in synthetic studies currently directed at unnatural opiate enantiomers. This route in its present form now renders either enantiomer of codeine, morphine and thebaine available in nearly 30% overall yield from m-methoxyphenethylamine with only 6-8 isolated intermediates. These results now offer, for the first time, a commercial source of medical opiates independent of the opium poppy. N-Cycloalkylmethylnorthebaine derivatives are also available in about 20% yield from the same starting material. That the medically valuable drugs nalbuphine and naltrexone can be directly synthesized in good overall yield from these northebaine derivatives has been demonstrated.

Bivalent agonist and antagonist opiate ligands containing either 2 natural or 1 natural and one unnatural opiate moieties were synthesized. The steroselectivity of potency enhancement observed further supported the hypothesis that opiate receptors are the vicinal recognition sites involved in the bridging of bivalent opiate ligands.

A number of unnatural enantiomers of opium-derived agonists and antagonists have been synthesized to study the mechanism of cough. The natural and unnatural enantiomers of codeine have been tritiated to high specific activity for further characterization of the binding sites which control this reflex.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 19235-05LC
Formerly
Z01 AM 19235-04LC

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization of Opiate Receptors Using Nonrigid Irreversible Inhibitors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	K. C. Rice	Research Chemist	NIDDK-LC
OTHERS:	A. E. Jacobson	Research Chemist	NIDDK-LC

COOPERATING UNITS *(if any)*

NIMH; (W. Klee, W. Simonds, R. Rothman, C. Pert, M. Herkenham)
Walter Reed Army Institute of Research, (J. Holaday)

LAB/BRANCH

Laboratory of Chemistry

SECTION

Medicinal Chemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our studies of the structure and function of opiate receptor subpopulations have continued using subpopulation specific irreversible inhibitors we developed earlier. Among these, BIT and FIT were shown to be mu and delta selective, respectively. In morphine tolerant rats selective upregulation of tritiated DADL binding sites was observed, and was confirmed by Scatchard analysis of tritiated DADL binding after FIT treatment of the membranes. Optimized conditions for tritiated bremazocine binding to kappa receptors have been defined in the rat and guinea pig brain. Treatment of rat and guinea pig brain sections with BIT and FIT followed by autoradiographic imaging with tritiated bremazocine revealed anatomical distribution of kappa sites. Optimized conditions to label mu, delta and kappa receptors have been utilized to determine receptor subtypes and density in the rat pituitary. Little if any mu and delta binding was observed, and the kappa binding was confined to the neural lobe where it was most dense in the external rim.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 19236-05LC
Formerly
~~Z01 AM19236-04LC~~

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization of Opioid Receptors Using Rigid Probes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P. I.	A. E. Jacobson	Research Chemist	NIDDK-LC
OTHERS	R. Lessor	Staff Fellow	NIDDK-LC
	K. C. Rice	Research Chemist	NIDDK-LC

COOPERATING UNITS *(if any)*

W. A. Klee; LGCB-NIMH-ADAMHA FOREIGN: none

LAB/BRANCH

Laboratory of Chemistry

SECTION

Medicinal Chemistry

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.9	0.9	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A series of relatively rigid molecules, from the endoethenooripavine family of opioids known to bind to opioid receptors from rat brain cerebrum homogenates and neuroblastoma-glioma hydride cells, have been found to act as irreversible binding ligands for the delta and mu opioid receptors. New synthetic methods for preparation of a simpler opioid family were explored as the base for potential kappa opioid affinity ligands.

PHS 6040 (Rev 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 AM 19237-04 LC

PERIOD COVERED: October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Topological Characterization of Opiate Receptors

PRINCIPAL INVESTIGATOR *(List other professional personnal below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	K. C. Rice	Research Chemist	NIDDK-LC
	T. R. Burke, Jr.	Staff Fellow	NIDDK-LC
	A. E. Jacobson	Research Fellow	NIDDK-LC

COOPERATING UNITS *(if any)*: W. Klee, NIMH, Bethesda, MD

LAB/BRANCH: Laboratory of Chemistry

SECTION: Medicinal Chemistry

INSTITUTE AND LOCATION: NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
  - ☐ (a1) Minors
  - ☐ (a2) Interviews
- ☐ (b) Human tissues
- ☒ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been temporarily discontinued.

PHS 6040 (Rev 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM19239-04LC

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Isolation and Characterization of the Antitrypanosomal Factor from P. fluoresens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. K. C. Rice Research Chemist NIDDK-LC

COOPERATING UNITS *(if any)*

LPD, NIAID (T. Mercado)

LAB/BRANCH

Laboratory of Chemistry

SECTION

Medicinal Chemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been temporarily discontinued.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 19241-05LC
Formerly
Z01 AM19241-04LC

PERIOD COVERED
October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Agonists and Antagonists for the Phencyclidine Receptor

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A.E. Jacobson	Research Chemist	NIDDK-LC
OTHER:	M. V. Mattson	Technician	NIDDK-LC
	K. C. Rice	Research Chemist	NIDDK-LC
	R. A. Lessor	Staff Fellow	NIDDK-LC
	A. Thurkauf	NRSA Fellow	NIDDK-LC

COOPERATING UNITS *(if any)*
Experimental Therapeutics Branch, NINCDS, NIH (Drs. T. O'Donohue, P. Contreras), Department of Pharmacology, University of Michigan Medical School (Drs. J. Woods).

LAB/BRANCH
Laboratory of Chemistry

SECTION
Medicinal Chemistry

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.4	1.9	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Metaphit, the first electrophilic affinity ligand specific for phencyclidine receptors, has been found to be a very useful compound for the study of phencyclidine receptors in various brain areas. Other ligands for the PCP receptor, including affinity ligands, have been synthesized for biochemical and pharmacological investigation.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 19243-04LC
Formerly
~~Z01 AM19243-03LC~~

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pyrrolidine Ant Toxins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Arnold Brossi	Visiting Scientist	NIDDK-LC
Others:	Wieslaw Gessner	Visiting Associate	NIDDK-LC

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Chemistry

SECTION

Section on Medicinal Chemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Lukes-Shorm dilactam used earlier to prepare pyrrolizidine-ant-toxins has now served to prepare (±)-trans-2-butyl-5-heptylpyrrolidine which is an ant alkaloid.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 19246-04LC
Formerly
Z01 AM 19246-03LC

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization of Opiate Receptors Using Positron Emission Transaxial Tomography

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	K. C. Rice	Research Chemist	NIDDK-LC
	T. R. Burke, Jr.	Senior Staff Fellow	NIDDK-LC
	A. Newman	Guest Worker	NIDDK-LC

COOPERATING UNITS *(if any)*

NIMH (C. Pert, A. Pert, N. Ostrowski), CC (S. Larson, R. Finn, M. Channing)

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Laboratory of Chemistry

SECTION

Medicinal Chemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We previously synthesized cyclofoxy, a fluoro analogue of the potent narcotic antagonist naltrexone, and showed the compound bound with high affinity to opiate receptors in vitro. This compound has now been tritiated to a specific activity of 16.4 Ci/mmol by mercuric oxide oxidation to the delta 15,16 dehydro derivative followed by palladium-catalysed reduction with carrier free tritium.

Methodology has also been developed for synthesis of high specific activity 3-acetylcyclofoxy labeled with positron emitting [fluorine-18] for PETT imaging studies. The method utilizes reactor-produced [fluorine-18] as its tetraethylammonium (TEA-F) salt in a SN2 displacement on a secondary triflate precursor. Typically, 45% of the [fluorine-18] activity can be converted to the reactive TEAF in a 70 min preparation. From this, 35% yield (decay corrected) of the [fluorine-18] 3-acetylcyclofoxy was obtained after HPLC purification with a specific activity of 25 Ci/mmol in a total synthesis time of 60 min. [Fluorine-18] 3-acetylcyclofoxy accumulation in opiate receptor rich brain regions of both rat and baboon was shown to be completely displaced by the active enantiomer of naloxone ((-)-naloxone) while the identical dose of the pharmacologically inert (+)-naloxone has no detectable effect.

Autoradiographic studies with tritiated cyclofoxy in rat brain slices have revealed that the compound labels a population of opiate receptors virtually identical to that labeled by naloxone. These and our previous results indicate that cyclofoxy is an excellent tool to study the physiological role of opiate receptors in living animals, and in humans using PETT imaging.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 19247-04LC
Formerly
Z01 AM 1924703 LC

PERIOD COVERED

October 1, 1985 through September 31, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Perhydrohistrionicotoxins

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principel Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Wieslaw Gessner	Visiting Fellow	NIDDK-LC
OTHERS:	Arnold Brossi	Visiting Scientist	NIDDK-LC

COOPERATING UNITS *(if any)*

J. Daly, NIDDK, NIH; E. X. Albuquerque, U. of Maryland, Baltimore.

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Section on Medicinal Chemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Two C-9 butylated ketolactams, obtained by alkylation of the "naked" lactam of the perhydrohistrionicotoxin series, were converted into pairs of epimeric alcohols. A C-9 dibutylated ketolactam was obtained as a by-product. The structure of one of the alcohols was verified by X-ray analysis.

C-9 alkylated perhydrotoxins represent a new class of models to study the acetylcholine receptor-channel complex.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 19249-03LC
Formerly
Z01 AM 19249-02LC

PERIOD COVERED

October 1, 1985 through September 31, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies on the Neurotoxin 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)

PRINCIPAL INVESTIGATOR *(List other professional personnal below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Arnold Brossi	Visiting Scientist	NIDDK-LC
OTHERS:	Wieslaw Gessner	Visting Fellow	NIDDK-LC

COOPERATING UNITS *(if any)*

C. W. Abell, Department of Biochemistry, University of Texas Medical Branch at Galveston; S. P. Markey, M LCS, NIH; J. P. N. Rosazza, U. of Iowa, Ames.

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Section on Medicinal Chemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

MPTP is a pseudosubstrate for the copper oxidase ceruloplasmin, and both $MPDP^+$ and MPP^+ are produced. Incubation of MPTP with horseradish peroxidase only provided MPTP N-oxide. Oxidation of $MPDP^+$ and MPP^+ with potassium ferricyanide afforded a dihydropyridone and a pyridone respectively. MPTP analogs derived from prodine-type analgesics were found to be devoid of neurotoxic properties. A series of deuterated MPTP-analogs was prepared and their inhibitory effects on MAO B measured.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 19250-03LC
Formerly
Z01 AM 19250-02LC

PERIOD COVERED

October 1, 1985 through September 31, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Chemistry and Metabolism of Qinghaosu, a Chinese Antimalarial Drug.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Arnold Brossi	Visiting Scientist	NIDDK-LC
OTHERS:	B. Venugopalan	Guest Scientist	NIDDK-LC

COOPERATING UNITS *(if any)*

D. Klayman, Walter Reed Research Institute; P. Buchs, SAPEC SA., Lugano, Switzerland; P. Trigg, SWG-CHEMAL, WHO, Geneva, Switzerland.

LAB/BRANCH

Laboratory of Chemistry

SECTION

Section on Medicinal Chemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

β-Dihydroquinghaosu-ethylether = Arteether, has been prepared from qinghaosu and characterized. Arteether is several times more potent than qinghaosu as an antimalarial and its oily solution will be clinically evaluated by im injection.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 19252-02LC
Formerly
Z01 AM 19252-01 LC

PERIOD COVERED

October 1, 1985 through September 31, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Synthesis of Morphine in Animal Tissue from Intermediates of its Plant Biosynthesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Arnold Brossi	Visiting Scientist	NIDDK-LC
OTHERS:	Kenner C. Rice	Research Chemist	NIDDK-LC
	Raymond Dumont	Visiting Fellow	NIDDK-LC

COOPERATING UNITS *(if any)*

Dr. Sidney Spector, Roche Institute of Molecular Biology, Nutley, New Jersey; V. Toome, Physical Chemistry Department, Roche, Nutley, NJ.

LAB/BRANCH

Laboratory of Chemistry

SECTION

Section on Medicinal Chemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Rats given thebaine and (+)-salutaridine, intermediates in the biosynthesis of morphine in poppy plants, produced significant levels of codeine and morphine in tissue of rats, suggesting that they may represent intermediates in the mammalian synthesis of morphine. Since both, (+)-salutaridine and (-)-thebaine were as pure as possible, it seems justify to exclude contamination of the two alkaloids with codeine or morphine as the source of mammalian codeine.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 19253-02LC
Formerly
Z01 AM 19253-01LC

PERIOD COVERED

October 1, 1985 through September 31, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Physostigmine and Analogs

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Arnold Brossi	Visiting Scientist	NIDDK-LC
OTHERS:	Bernhard Schönenberger	Visiting Fellow	NIDDK-LC
	Bernhard Witkop	Chief, LC	NIDDK-LC

COOPERATING UNITS *(if any)*

Prof. E. X. Albuquerque, University of Maryland, Baltimore; Dr. R. Ray, Pharmacology Branch, US Army Medical Research, Aberdeen Proving Ground; S. Rapoport, NIA LN, NIH.

LAB/BRANCH

Laboratory of Chemistry

SECTION

Section on Medicinal Chemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Anticholinesterase activity of analogs of natural (-)-physostigmine, was measured. (-)-N-Methylphysostigmine was found to be much more potent, whereas unnatural (+)-physostigmine was about 125 times less potent. Fragmentation of (-)-physostigmine in refluxing hexanol afforded (-)-eseroline quantitatively. X-ray diffraction analysis showed rubreserine to contain equal parts of o-quinone and its zwitterion.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 19254-02LC

PERIOD COVERED

October 1, 1985 through September 31, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Peripheral and Central Effects of (-)- and (+)-Mecamylamine

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Arnold Brossi	Visiting Scientist	NIADDK-LC
OTHERS:	B. Schönenberger	Visiting Fellow	NIADDK-LC

COOPERATING UNITS *(if any)*

E. X. Albuquerque, University of Maryland, Baltimore

LAB/BRANCH

Laboratory of Chemistry

SECTION

Section on Medicinal Chemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated.

Schönenberger, B., Brossi, A. Geroge, C. and Flippen-Anderson, J.L.: Preparation of Optically Active Secondary Amines by Thermal Decomposition of (Methylbenzyl) urea Analogs: Absolute Configuration of (+)-and (-)-Mecamylamine, Helv. Chim. Acta 69, 283-287 (1986).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 19255-02LC
Formerly
Z01 AM 19255-01LC

PERIOD COVERED

October 1, 1985 through September 31, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

8-Aminoquinoline antimalarials

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Arnold Brossi	Visiting Scientist	NIDDK-LC
OTHERS:	Wieslaw Gessner	Visiting Fellow	NIDDK-LC
	B. Venugopalan	Guest Scientist	NIDDK-LC

COOPERATING UNITS *(if any)*

H. Rupp, Hoechst India, Research Institute Mulund; I. Landau, Laboratoire des Vers, Paris, C. W. Abell, U. Texas Medical Center, Galveston.

LAB/BRANCH

Laboratory of Chemistry

SECTION

Section on Medicinal Chemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Removal or blocking the primary amino group of primaquine is accompanied with a complete loss of antimalarial activity (-)- and (+)-Primaquine showed different cellular toxicity in vitro. Photooxidation of N-acylated primaquine afforded o-quinones.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 19256-01LC

PERIOD COVERED

October 1, 1985 through September 31, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mammalian Alkaloids

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Arnold Brossi	Visiting Scientist	NIDDK-LC
OTHERS:	B. Schönenberger	Visiting Fellow	NIDDK-LC
	C. Schoenberger	Guest Scientist	NIDDK-LC

COOPERATING UNITS *(if any)*

H. Thomas, Department of Physiology, University of Ulm, West Germany; C. W. Abell, University of Texas Medical Center, Galveston; J. Flippen-Anderson, Naval Research Laboratory, Dept. of the Navy, Washington, D.C.

LAB/BRANCH

Laboratory of Chemistry

SECTION

Section on Medicinal Chemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Mammalian 1-carboxy sustituted tetrahydroisoquinolines are represented by salsoline-1-carboxylic acid and 3',4'-deoxynorlaudanosoline carboxylic acid produced in phenylketonurics. Attempts have been made to prepare the optical isomers of these carboxylic acids and to determine their configuration by X-ray analysis. Configurational assignments were made for (-)- and (+)-salsoline-1-carboxylic acid and for (-)- and (+)- salsolinol-1-carboxylic acids prepared from the former by O-demethylation. Racemic and optically active salsoline and isosalsoline were prepared to study enzymic O-demethylation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 19257-01LC

PERIOD COVERED

October 1, 1985 through September 31, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

1-Phenylisoquinolines

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Arnold Brossi	Visiting Scientist	NIDDK-LC
OTHERS:	B. Venugopalan	Guest Scientist	NIDDK-LC

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Chemistry

SECTION

Section on Medicinal Chemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Several 1-phenyl-substituted 1,2,3,4-tetrahydroisoquinolines and 1-phenylisoquinolines were made by conventional chemistry. Reaction of a representative 1-phenylisoquinoline with sodium nitrite in acetic acid afforded a methyl ether analog of the natural product necatorone.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 19401-21 LC
Formerly:
Z01 AM 19401-20 LC

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Natural Products as Agonists, Antagonists, Desensitizers & Probes for Receptors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Dr. Bernhard Witkop Chief LC, NIDDK

Cooperating Units: I.L. Karle, U.S. Naval Res. Lab., Wash.D.C.; E.X. Albuquerque, C. Spivak & M.P. Blaustein, Univ. MD Med. Sch.; T. Gund, N.J. Inst.Tech.,Newark,N.J.; Prof. Gabor Fodor, Dept.Chem.,W.Va.Univ.; R. Aronstam, Univ.GA. Foreign: Boris Khodorov, Vishnevsky Inst. Surgery, Moscow; O. Yonemitsu, Y. Kanaoka & T. Iwakum, Univ.Hokkaido; E. Gössinger, Univ.Vienna,Austria; E.M. Kosower, Tel-Aviv Univ.; Shin-Chi-yi, Univ. Peking.

LAB/BRANCH
Laboratory of Chemistry

SECTION
Section on Metabolites

INSTITUTE AND LOCATION
NIH, NIDDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.5		.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Chief Investigator - largely with extramural support - has kept up a widely diversified program, international and interdisciplinary in character, involving binding studies of agonists, electrophysiology of ion flux, photochemistry of psychoactive drugs, modeling of nicotinic and muscarinic agonists, consultation on protective measures against organophosphorous agents--and support function for a clinical program on degenerative diseases of the rain. In addition the Chief Investigator is active in international scientific exchange and collaboration with most countries of Western Europe, China, Japan and Taiwan. He is Editor of FEBS Letters (Federation of European Biological Societies) for North America, Member of the Paul Ehrlich Foundation in Frankfurt, Germany, and as (honorary) Member of Academics and Learned Societies of Europe and Japan participates in the formulation of research aims and policies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 19402-13 LC
Formerly:
Z01 AM 19402-12 LC

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Interferon Induction and Action. The Antiviral Activity of Nucleoside Analogs

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Paul F. Torrence	Research Chemist	NIDDK-LC
Others:	Alice Wong	Technician	NIDDK-LC
	David Alster	National Research Service Award Fellow	NIDDK-LC
	Yukio Kitade	Visiting Fellow	NIDDK-LC
	Danute Brozda	Visiting Fellow	NIDDK-LC

COOPERATING UNITS *(if any)*
FOREIGN: J.-L. Imbach, U. Montpellier, France; W. Dawson, U. Cal.; J. Mond, USUHS, C. Altona, Univ. Leiden, Netherlands; W. Pfleiderer, U. Konstanz, W. Germany

LAB/BRANCH
Laboratory of Chemistry

SECTION
Section on Metabolites

INSTITUTE AND LOCATION
NIH, NIDDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	2	1

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Interferon-induced enzyme activities such as the oligo(2' 5')adenylate synthetase, the 67K dalton protein kinase and oligo(2' 5') A phosphodiesterase are investigated with a goal of understanding their role in the action of interferon, the induction of interferon by double-stranded RNA and, perhaps, control of cell growth and differentiation. Analogs of the mediator of interferon action, 2-5A, are synthesized in order to define the relationship between oligonucleotide structure and binding to and activation of the 2-5A dependent endonuclease with the eventual goal of designing useful chemotherapeutic agents based on this system.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 19603-10 LC
Formerly
Z01 AM 19603-09 LC

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Histidine Analogues

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	Louis A. Cohen, Chief,	Section on Biochemical Mechanisms,	LC, NIADDK
Others:	Kenneth L. Kirk	Research Chemist	NIDDK-LC
	Kazuyuki Takahashi	Visiting Associate	NIDDK-LC
	Virender Labroo	Visiting Associate	NIDDK-LC
	Minh Truong	Biological Aide	NIDDK-LC

COOPERATING UNITS *(if any)*

P. Dannies, Dept. of Pharmacology, Yale Medical School, New Haven, Conn.
G. Feuerstein, Dept. of Pharmacology, USUHS
E. De Clercq, Louvain, Belgium
H. Kimoto, Nagoye, Japan

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Laboratory of Chemistry

SECTION

Section on Biochemical Mechanisms

INSTITUTE AND LOCATION

NIH, NIADDK, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.1	1.4	0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

TRH Analogs: In addition to governing the release of thyrotropin and prolactin in the pituitary gland, TRH (L-pyroglutamyl-L-histidyl-L-proline amide) is known to possess a wide variety of effects on both the central nervous system (CNS) and the cardiovascular system (CVS). TRH has shown promise for use in the treatment of shock, as an analeptic and antidepressant, and as a promoter of the regeneration of injured spinal cord. However, the great variety of its biological effects presents a serious drawback to its use as a specific drug. Our early studies with synthetic analogues of TRH (involving modification of the imidazole ring of histidine) has suggested that the peptide hormone elecits each of its physiological responses at a different receptor and that appropriate analogues may achieve some of the desired specificity of action. In contrast to the natural peptide, 4-fluoro-Im-TRH does not bind to rat pituitary cells in vitro and does not release prolactin from them; such results would immediately suggest the analogue to be nonfunctional. When it is microinjected directly into rat brain, however, it effected significant increases in heart rate and blood pressure. We have now prepared and tested by systemic injection a variety of other analogues on the CVS and endocrine systems of conscious rats. 4-TFM-TRH, 2-TFM-TRH and 4-nitro-TRH were as potent as TRH in increasing mean arterial pressure, pulse pressure and heart rate at both 1 mg/kg and 5 mg/kg doses. At the same time, 4-TFM-TRH and 2-TFM-TRH were 4-5 times more potent than TRH in increasing plasma prolactin; 4-nitro-TRH, on the other hand was totally devoid of prolactin-releasing activity. Nor-Val-TRH was as effective as TRH (at either dose) in increasing plasma prolactin but, surprisingly, was devoid of any CVS activity.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Formerly

PROJECT NUMBER

Z01 DK 19604-16 LC
Z01 AM 19604-15 LC

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

General Principles of Enzyme Catalysis and Simulation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. Louis A. Cohen, Chief, Section on Biochemical Mechanisms, LC, NIADDK

Other: Michael King Guest Worker GWU

COOPERATING UNITS *(if any)*

Eugene Man, University of Miami
Yoshio Ueno, Nagoya, Japan
Wieslow Antkowiak, Poznan, Poland

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Laboratory of Chemistry

SECTION

Section on Biochemical Mechanisms

INSTITUTE AND LOCATION

NIH, NIADDK, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.3	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In order to account for the remarkable catalytic power of enzymes, it is generally considered that the activation free energy is contributed both by binding of the substrate to the enzyme (step 1) and by chemical manipulation of the bound substrate (bond-making and breaking, step 2). Popular opinion holds that most of the activation energy is supplied in step 2: We have proposed, however, that the overall catalytic process can be explained more reasonably if it is assumed that the first step (binding) contributes a more significant, and sometimes major, share of the activation energy. To support this theory, we have synthesized a large variety of test-tube models which simulate the bound substrate by being frozen into a single, favorable conformation and by having the interacting groups brought into the closest possible juxtaposition (stereopopulation control). These compounds undergo intramolecular reactions at rates comparable to those catalyzed by enzymes, sometimes even too fast to measure. The protein raises both the entropic and enthalpic components of the substrate by binding it in a single, rigid conformation.

Our original theory proposed that the principal sources of free energy increase during binding were conformational freezing, desolvation, electronic deformation, etc. Our new studies with tryptophan analogs have provided yet another factor which we had not considered originally: in those cases in which a substrate is capable of tautomeric equilibrium, the enzyme may be able to stabilize (by binding alone) the thermodynamically unfavorable tautomer (Tenutautomer). This simple event would necessarily increase the free energy content of the bound substrate and serve as "activation." We have already proven the reality of this phenomenon for tryptophan by demonstrating the potent inhibitory and sterospecific properties of TENUTAUTOMER ANALOGS; We are presently involved in the design, synthesis and testing of other stable tenutautomer analogs.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 19605-10 LC
Formerly
Z01 AM 19605-09 LC

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Chemistry of Substituted Imidazoles

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. Louis A. Cohen, Chief, Section on Biochemical Mechanisms, NIADDK, LC

Others:	Robert Jerussi	Guest Worker (FDA)	NIADDK-LC
	Stuart Cohen	Guest Worker (EPA)	NIADDK-LC
	Virender Labroo	Visiting Associate	NIADDK-LC
	Minh Troung	Biological Aide	NIADDK-LC
	Kazuyuki Takahashi	Visiting Associate	NIADDK-LC

COOPERATING UNITS *(if any)* H. Kimoto, Industrial Res. Inst., Nagoya, Japan; R. Henkin, Georgetown Univ. Hosp., Wash. D.C.; E. De Clercq, Louvain Univ., Belgium; A. Shanzer, Rehovot, Israel; W. Nagai, Nagoya, Japan; G. Feurstein, USUHS; D. Lozovsky, LCS, NIMH.

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SECTION
Section on Biochemical Mechanisms

INSTITUTE AND LOCATION
NIH, NIADDK, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.2	1.5	0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Ring-trifluoromethylated imidazoles show the unique property of losing hydrogen fluroride above pH8 to form metastable difluoroduzafulvenes, which then react with any available nucleophile to form new covalent bonds. Such intermediates, derived from trifluoromethylhistamine or histidine, may be able to serve as covalent affinity labels for specific binding sites, both in vitro in vivo. It would be desirable, therefore, to have available a series of trifluoromethyl analogues with a range of reactivities, and to be able to correlate reactivity with some substituent parameter. Our discovery of a simple photochemical method for the trifluoromethylation of imidazoles has made available a large series of analogues for study. We have now found that the reactivities of some members of the group can be correlated with the special electronic effects of certain substituents (capable of hyperconjugation or back-bonding). Computer analysis of reactivity data for a series of trifluoromethylimidazoles has provided a linear free energy relationship in which log k_r correlated with both inductive and resonance components of the respective substituents. According to computer-based predictions, the fluoro group would provide the ideal combination of acidity and reactivity under physiological conditions. We have, therefore, developed procedures for sequential photochemical introduction of fluorine and trifluoromethyl into imidazoles and have verified the predicted reactivities. We are now involved in the preparation of peptide hormones containing these substituents. Photochemical introduction of the trifluoromethyl group has been found practical for more complex imidazoles and studies are under way for the synthesis of the trifluoromethyl analogue of the anti-ulcer drug, cimetidine.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 19606-10 LC
Formerly
Z01 AM 19606-09 LC

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Halogenated Biogenic Amines in Biochemistry and Pharmacology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: Kenneth L. Kirk, Research Chemist, NIDDK, LC

Other: David Furlano, Guest Worker (NRSA Fellow)
Adeboye Adejare, Visiting Fellow, NIDDK,LC
Kenneth A. Jacobson, Staff Fellow, NIDDK,LC
George Chen , Guest Worker, NIDDK, LC

COOPERATING UNITS *(if any)*
J. Daly, C.R. Creveling, F. Gusovasky (LBC, NIDDK); M. Channing, S. Larson (CC, Dept. of Nuclear Medicine); R. Phillips, University of Georgia): E. Stone (New York University); A. Shanzer, K. Muszkat (Weizman Institute, Israel).

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SECTION
Section on Biochemical Mechanisms

INSTITUTE AND LOCATION
NIH, NIADDK, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.2	3.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Biogenic amines play key roles in neurotransmission, metabolism, and in control of various physiological processes. Ring-fluorinated analogs have proved to be powerful tools for the study of the mechanisms of transport, storage, release, metabolism and modes of action of these amines since they simulate the geometries of the natural compounds so well. By virtue of its very small size and high electronegativity, fluorine should be a very favorable replacement for hydrogen in these analogs. Some years ago, we developed novel methods for the introduction of fluorine into organic molecules and have applied these methods to the syntheses of a wide variety of biogenic amines with fluorine at various ring positions. The biological properties and usefulness of these ring-fluorinated biogenic amines has proved to be extremely rewarding and continue to find application in a multitude of studies. Perhaps the most significant finding, to date, is that 6-fluoronorepinephrine is a pure alpha-adrenergic agonist, while the 2-fluoro epinephrine is a pure beta-adrenergic agonist. Various explanations for the role of fluorine in creating such specificity have been considered and discarded. We now propose that a critical dipole-dipole repulsion between the benzyllic hydroxyl group and fluorine in the 2- or 6-position confers side chain conformational preference favorable for interaction with the beta- and aplph-adrenergic receptor, respectively. The discovery of increased potency in a fluorinated alpha-adrenergic agonist has both practical and theoretical importance. Chemo-enzymatic methods have been used to prepare radiolabeled fluorinated analogs as well as new analogs as potential prodrugs for melanoma chemotherapy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
ZO1 DK 19607-04 LC
Formerly
ZO1 AM 19607-03 LC

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Chemistry, Biochemistry and Pharmacology of Bioindole Analogs

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. Louis A. Cohen, Chief, Section on Biochemical Mechanisms, LC, NIDDK

Other: Rita Labroo Guest Worker GWU

COOPERATING UNITS *(if any)*

Edith Miles, LBP, NIADDK

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Laboratory of Chemistry

SECTION
Section on Biochemical Mechanisms

INSTITUTE AND LOCATION
NIH, NIADDK, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.0	.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The mechanisms of hydrolysis of the 2-halotryptophans at low pH have now been fully elucidated and reveal the involvement of intramolecular proton transfer in the conversion of the stable indole to the labile indolenine tautomer. An enzyme carboxyl group should also promote indolenine formation, suggesting the indolenine to be the true substrate for certain tryptophan enzymes.

The first conclusive support for this concept is found in the demonstration that 2,3-dihydro-L-tryptophan and oxindolyl-L-alanine, analogs of the indolenine tautomer of tryptophan (tetrahedral carbon at C-3), are potent competitive inhibitors of tryptophan synthase and tryptophanase. Furthermore, the two enzymes show opposing specificity for the C-3 diastereoisomers of 2,3-dihydro-L-tryptophan, suggesting that these enzymes catalyze their reactions via enantiomeric indolenine intermediates.

Fluorine-19 nuclear magnetic resonance and differential absorption spectroscopy have been used to study the binding and reactions of the D and L isomers of 5-fluorotryptophan, tryptophan and of (3S)- and (3R)-2,3- dihydro-5-fluorotryptophan. Tryptophan synthase specifically and tightly binds the (3S) diastereoisomer of both 2,3-dihydro-5-fluoro-D-tryptophan and 2,3-dihydro-5-fluoro-L-tryptophan, whereas it binds 5-fluoro-D- tryptophan more tightly than 5-fluoro-L-tryptophan. Unexpectedly, we find that the D and L isomers of 5-fluorotryptophan, tryptophan, and (3S)-2,3dihydro-5-fluorotryptophan are slowly interconverted by isomerization reactions. These isomerization reactions are much slower than the β-replacement and β-elimination reactions catalyzed by tryptophan synthase. Since pyridoxal phosphate itself slowly catalyzes many reactions of amino acids in model systems, our results raise the interesting question of whether tryptophan synthase itself serves a catalytic role in these slow reactions or whether the enzyme simply binds the substrate and pyridoxal phosphate stereospecifically and thus promotes the intrinsic catalytic activity of pyridoxal phosphate. Our results further define the stereochemistry of the substrate binding site of tryptophan synthase.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 19608-03 LC
Formerly:
Z01 AM 19608-02 LC

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Functionalized Congeners of Bioactive Compounds

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	K. Jacobson	Staff Fellow	NIDDK-LC
Other:	K. Kirk	Research Chemist	NIDDK-LC
	E. Pijl	Guest Worker	NIDDK-LC

COOPERATING UNITS *(if any)* J. Daly (NIDDK), D. Ukena (NIDDK), B. Fredholm (Karolinska Inst.), J. Carney (U. Oklahoma), H. Fales (NHLBI), P. Churchill (Wayne State Univ.), R. Olsson (Univ. So. Fla.), G. Stiles (Duke Univ.), K. Seamon (FDA), G. Aurbach (NIDDK), P. Marangos (NIMH), M. Williams (CIBA-GEIGY)

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Laboratory of Chemistry

SECTION
Section on Biochemical Mechanisms

INSTITUTE AND LOCATION
NIH, NIDDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.4	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Recent work in our laboratory and in others has demonstrated that certain drugs may be attached to well-defined "carrier" molecules and still retain the ability to bind to the receptor site and effect biological activity. This synthetic strategy for the attachment of drugs to carriers is termed the "functionalized congener" approach. The "carrier" molecule may be many times larger than the parent drug; indeed there is practically no maximum size limitation for a fully potent analog. Unlike the prodrug approach or the immobilization of drugs for slow release, the "functionalized congener" approach is designed to produce analogs for which no metabolic cleavage step is necessary for activation. Moreover, the attachment of the drug to a "carrier" such as a peptide may result in the enhanced affinity at an extracellular receptor site, and an improvement in the pharmacological profile of the parent drug.

The extracellular adenosine receptor has a modulatory role in the nervous, circulatory, endocrine, and immunological systems. The prospect of harnessing these effects specifically for therapeutic purposes is attractive, but efforts have not met with much success in the past.

The functionalized congener approach has been applied to the adenosine receptor to produce analogs of agonists and antagonists which have promise as therapeutic agents and as receptor probes. In the antagonist series new analogs which combine potency, water solubility, and A_1-adenosine receptor selectivity in the same compound are now being evaluated in in vivo testing.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 19609-02 LC
Fomerly
Z01 AM 19609-01 LC

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Determination of Amines and Amine Metabolites in Biological Samples

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. Kenneth L. Kirk, Research Chemist, NIDDK, LC

Other: Kenneth A. Jacobson, Staff Fellow, NIDDK, LC

COOPERATING UNITS *(if any)*
M. Linnoila, NIAAA, NIH
T. Marshall, NIAAA, NIH
R.T. Brown, NINCDS, NIH
F. Gusovsky, LBC, NIDDK

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SECTION
Section on Biochemical Mechanisms

INSTITUTE AND LOCATION
NIH, NIDDK, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The importance of biogenic amines in neurotransmission, metabolism, and in control of various physiological processes has spurred intense interest in the development of precise and sensitive methods for the quantitation of these amines and their metabolites present in cebrospinal fluid, plasma and urine.

We have developed precise, operationally simple procedures for the analyses of certain of these substances based on HPLC separation using highly sensitive electrochemical detection, coupled with the use of internal standards for accurate quantitation. The utility of the approach has been extended greatly by selective and efficient acylation of the endogenous amines which permits ready isolation from the biological sample. Use of an electroactive acylating reagent further extends this approach to the accurate detection of amines which, themselves, are not amenable to electrochemical detection (e.g., histamine and phenethylamine).

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 19801-41 LC

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Service Functions and Instrumentation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Dr. David F. Johnson Chief, Micro. Ser. & Instr. NIADDK-LC

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Chemistry

SECTION

Microanalytical Services and Instrumentation

INSTITUTE AND LOCATION

NIH, NIDDK, Bethesda, Md. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Project transferred to the Laboratory Analytical Chemistry

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 19803-13 LC

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Applications of NMR in Biochemical and Biological Systems

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. Dr. Herman Yeh Research Chemist NIDDK/LC

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Chemistry

SECTION

Microanalytical Services and Instrumentation

INSTITUTE AND LOCATION

NIDDK, NIH, Betheda, Md. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the spece provided.)*

Project transferred to the Laboratory of Analytical Chemistry

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 19804-13 LC

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Development of Methods and Materials for the Study of Medical Problems

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. Calvin M. Foltz Research Chemist NIDDK, LC

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Chemistry

SECTION

Microanalytical Services and Instrumentation

INSTITUTE AND LOCATION

NIDD, NIH, Betheda, Md. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Project transferred to the Laboratory of Analytical Chemistry

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 AM 19806-11 LC

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Nature of Steroid-Receptor Interactions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. S. Stoney Simons, Jr. Research Chemist NIDDK, LC

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Chemistry

SECTION
Microanalytical Services and Instrumentation

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Md., 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Project transferred to the Laboratory of Analytical Chemistry

ANNUAL REPORT OF THE LABORATORY OF CELL BIOLOGY AND GENETICS
NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

The Laboratory of Cell Biology and Genetics carries on a broad program of investigation into hormone and transmitter secretion and the molecular events regulating these processes. Three specific tissues are used: chromaffin cells, which secrete adrenaline, ATP and endogenous opiates; pancreatic beta cells, which secrete insulin; and the frog neuromuscular junction, in which acetylcholine is the principal secreted substance.

Membrane fusion is a key event in numerous biological processes, including neurotransmission and exocytosis, and may depend on calcium and other ions and factors. Much of our work this year has been devoted to biological signals which regulate this process in chromaffin and B cells, as well as to studies on the calcium binding protein synexin. At present we consider synexin, and the other approximately one dozen closely related proteins, to be likely mediators of membrane contact and fusion during exocytosis.

Synexin mediates the contact and fusion of chromaffin granules to one another in the presence of calcium and arachidonic acid. Both calcium and arachidonic acid are present in increased amounts in secreting chromaffin and B. cells, and the fusion products closely resemble the compound or piggyback exocytosis figures noted in both cell types following fusion. Furthermore, both synexin activity in vitro and secretion in vivo are blocked by low concentrations of trifluoperasine and promethazine. These phenothiazines are quite distinct otherwise in terms of effects on calmodulin or as local anesthetics.

We are now devoting strong efforts to cloning the gene for synexin. A number of libraries have been prepared in the lambda gtl1 expression vector system, and a large number of clones derived which express proteins reacting with a number of independently derived monoclonal antibodies and to a polyclonal antibody. Populated libraries have been isolated from bovine adrenal medulla and from bovine liver. A 547 bp clone has been completely sequenced and a number of revealing properties deduced from analysis of this partial sequence.

Inasmuch as we plan to prepare mutant synexins by the process of site-directed mutagenesis, we have also devoted substantial efforts to devising assays for synexin function. One of these has been an assay of direct membrane fusion using chromaffin granule ghosts with self-quenching concentrations of FITC dextran sealed within. Upon fusion, the FITC-dextran dequenches and the resulting signal indicates fusion. Leakage is controlled by inclusion of an anti-FITC antibody in the medium. While this fusion system is partially sensitive to calcium concentration, we have also determined that the fusion reaction is also sensitive to protons. They may be relevant to secretion since an increasing amount of evidence (vide infra) suggests that cells may become relatively acidotic during the secretory process. The proton-sensitive component may be partially dependent on proteins in the membrane, while the calcium sensitive component may be more dependent on acidic/neutral phospholipids in the membrane.

Signals regulating membrane fusion may also include protein kinase C substrates, action of phospholipase C to produce inostoltrisphosphate (IP3), and cyclic nucleotide metabolism. Protein kinase C has attracted our attention because the phorbol ester TPA can potentiate Ca^{2+}-evoked catecholamine release from permeabilized chromaffin cells and Ca^{2+}/A23187-treated intact chromaffin cells. The only receptor known for TPA is the protein kinase C, and we have therefore proceeded to purify the adrenal medullary enzyme and compare some of its properties with secretory phenomena in chromaffin and B cells. Indeed, no endocrine protein kinase C had been purified to homogeneity until this effort, and a detailed study revealed distinct differences to the previously studied enzymes from other tissues. The calcium sensitivity in the presence of diolein or TPA, however, was lowered only to the low micromolar range, as noted previously with the crude enzyme. The operational meaning of this in terms of the true calcium concentration during secretion is yet to be determined, although the recent literature is consistent with concentrations of free calcium in the 10-50 M range, depending on the choice of detector.

Diolein is of course derived from a phospholipid treated with phospholipase C. Conventionally, the lipid is usually phosphatidylinositol and its polyphosphate derivitives. Indeed, chromaffin cells treated with acetylcholine both secrete catecholamines and ATP and generate IP3. Hitherto it has been known that secretion was nicotinic while the specific phospholipase C in other systems was muscarinic. Indeed, only muscarine, a non-secretagogue, could generate IP3 in chromaffin cells. However, a detailed kinetic analysis revealed that if nicotine and muscarine were administered within one minute of one another secretion by nicotine was potentiated. This effect seems to be correlated with IP3 generation, and to be likely due to mobilization of intracellular calcium.

Cyclic AMP in chromaffin cells is generated during the secretory process, but seems to be mainly involved in housekeeping chores such as activating tyrosine hydroxylase. However, in B cells the matter is quite different. In this case cAMP appears to increase spike frequency, activating the unloading of cytosolic calcium and inhibiting the [Ca] activated K^+ channel. In addition, cAMP and drugs that elevate cAMP such as forskolin and cholera toxin enhances cell-to-cell coupling, as measured with dyes and experiments using two microelectrodes.

As alluded to above, we have also studied the influence of intracellular pH on secretion, as well as the processes which regulate intracellular pH in both chromaffin and B cells. In chromaffin cells almost all physiological secretagogues also induce intracellular acidification, and the pH(i) can be controlled by manipulation of the Na^+/N^+ antiporter. The exact relation of these pH changes to secretion in terms of cause or effect will doubtless be worked out over the course of the coming year. But, in the B cell intracellular pH has been found to have influence on both ionic channels in the membrane and on secretion, per se. In the single mouse islet, ammonium chloride increases cytoplasmic pH, as expected. However, the consequence was the

K^+ permeability was activated and the glucose-induced electrical activity was suppressed. Furthermore, insulin secretion was also suppressed. The inhibition of release seems to be based on the likelihood that increased K^+ permeability reduces Ca^{2+} influx through voltage-sensitive Ca channels, resulting in suppression of secretion. However, methylamine, an ammonia analogue, was able to block insulin release without affecting electrical activity of single perifused islets at 2 and 6 mM. At 10 mM, however, both insulin release and electrical activity wre suppressed. These data thus indicate that intracellular pH may affect secretion and membrane activity differentially, a conclusion consistent with previous findings from this Laboratory regarding dissociation of membrane activity and secretion in B cells.

Finally, considerable effort has been expended in determining the role of ascorbic acid in chromaffin granule assembly. The initial hint on the involvement of ascorbic acid in chromaffin cell function came from our finding that ascorbic acid treatment of chromaffin cells in culture caused an enhanced synthesis of norepinephrine from tyrosine. We found that this effect could also be detected in digitonin permeabilized chromaffin cells, and in intact chromaffin granules when dopamine was the substrate. This result was consistent with the possibility that the site of action was the biosynthetic enzyme DBH. Indeed, ascorbate can activate purified DBH by reducing the copper ions attached to the enzyme. However, the actual mechanism of ascorbate activation of DBH in intact granules proved to be quite different. First, ascorbic acid was found to be profoundly impermeant to the granule membrane. Since DBH is on the inside of the granule direct access of ascorbate to DBH seemed denied. However, we and others finally determined that ascorbate can transfer its electrons across the membranes via the cytochrome b562, described earlier in this section. In addition, while other reducing agents can substitute for ascorbate in the assays with purified DBH, only ascorbate will donate electrons to DBH when it is in its native intragranular environment. The meaning of this finding may prove to be seminal in terms of finally understanding the biological actions of ascrobate. Connective tissue effects are quite long term in onset, while action on the chromaffin cell system are quite acute. It is thus possible that the chromaffin cell system may prove to be the elusive site of action for ascorbate in what is termed loosely "stress" situations.

The basis characteristics of all endocrine organs involve a capillary network with the endocrine cells packed in corn-cob fashion on the capillary tubes. We noticed that in chromaffin cell cultures chromaffin cells were always attached to the bottom of the well by a flat interdigitating cell. Subsequent analysis revealed that this cell was the remnant of the capillary network, and that it could be isolated and passaged virtually endlessly. These endothelial cells have now been patented by members of our laboratory and the NIH as a source of a number of clotting factors, including Factor VIII:C, and we have used them to examine the nature of the interaction between chromaffin cells and endothelial cells. Since freshly prepared chromaffin cells often have endothelial cells firmly adherent, we chose to examine the interaction of the tumor cell analogue, PC-12, with cultured bovine endothelial cells. We found that PC-12 cells rapidly (t1/2 ca. 30 min.)

adhered specifically to endothelial cells and not to intercellular matrix. Furthermore, 1 M urea extracts of either PC-12 or endothelial cells, comprising mainly blebs of plasma membrane, inhibited this interaction. Finally, we found that proteases blocked the activity of the urea extracts, leading us to conclude that interaction seemed to depend on a protein. Surprisingly, when the co-cultures were left for longer times (1 week), the PC-12 cells were found to stop cell division and assume a more differentiated, glandlike character. Studies on a variety of protooncogenes seem to bear out this qualitative observation.

We conclude this report with what is perhaps the most promising technical advance in cell biology. Using the rapid freezing technique and the elemental imaging microscope we have been able to determine elemental compositions of chromaffin granules and chromaffin cell cytosolic compartments, before and after biochemical isolation. Taking advantage of the fact that chloride passively distributes with electrical potentials we have also been able to measure the electrical potentials across accessible membrane surfaces in the entire cell. As anticipated, in the resting cell, the plasma membrane potential is negative with respect to the extracellular solution and that the chromaffin granule is positive inside with respect to the cytosol. Changes occur during stimulation. Furthermore, the granule contents of potassium and calcium were found to vary quite substantially compared to biochemically purified granules: much more K^+ and Ca^{2+} were found *in vivo* than *in vitro*. This may relate to the fact that the chemiosmotic properties of granules within the cells are quite different from those of isolated granules.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 AM 21003-18 LCBG

PERIOD COVERED
October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biomechanics and related subjects

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: C. W. McCutchen Research Physicist LCBG:NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Cell Biology and Genetics

SECTION
Cell Biology and Biochemistry

INSTITUTE AND LOCATION
NIH:NIDDK, Bethesda, Md. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been temporarily discontinued.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 AM 21005-19 LCBG

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Histochemistry: Principles, Methods and Applications

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
P.I.: N. Feder Medical Officer (Research) LCBG:NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Cell Biology and Genetics

SECTION
Section on Biophysical Histology

INSTITUTE AND LOCATION
NIH,NIDDK,Bethesda,Md. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been transferred to the Laboratory of Analytical Chemistry.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 21,008-20 LCBG

PERIOD COVERED
October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cytogenetics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	J.H. Tjio	Chief, Section on Cytogenetics	LCBG NIDDK
Others:	B.J. White	Medical Officer (Research)	LCBG NIDDK

COOPERATING UNITS *(if any)*
Laboratory of Microbiology, Albany Medical College, Albany, NY (E.S. Raveché); University of California, Berkeley (G. Brecher); Brookhaven National Laboratory, Long Island, NY (E.P. Cronkite).

LAB/BRANCH
Laboratory of Cell Biology and Genetics

SECTION
Cytogenetics

INSTITUTE AND LOCATION
NIH, NIDDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)
- ☐ (a) Human subjects
  - ☐ (a1) Minors
  - ☐ (a2) Interviews
- ☐ (b) Human tissues
- ☒ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

1. H.Y. identification in mouse and fish.

2. Immune reactivity following syngeneic bone marrow transfusions.

3. Genetic analysis of abnormal hyperdiploid spleen cells of NZB mice.

4. Murine hemopoietic stem cells following bone marrow transfusions.

5. Sex reversal in fish.

6. Human cytogenetic studies of patients with congenital and developmental disorders as well as studies of mutagen-induced chromosomal abnormalities are continuing.

7. Application of differential staining techniques and continued improvement of established methods are basic to all of the projects conducted in the laboratory.

Formerly Z01 AM 21,008-19 LCBG

PHS 6040 (Rev 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 21,009-19 LCBG

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cytogenetic Investigations

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B.J. White	Research Medical Officer Section on Cytogenetics	LCBG, NIDDK
Other:	J.H. Tjio	Chief Section on Cytogenetics	LCBG, NIDDK

COOPERATING UNITS *(if any)*
Inter-Institute Genetics Program, NIH; Laboratory of Neurosciences, NIA, NIH (M. Shapiro); Clinical Neurogenetics Branch, NIMH, NIH (L. De Lisi); Genetics Department, Children's Hospital National Medical Center (K. Rosenbaum).

LAB/BRANCH
Laboratory of Cell Biology and Genetics

SECTION
Cytogenetics

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Cytogenetic studies of patients with genetically determined or influenced disorders are conducted. Constitutional and secondary chromosomal variations and their relationship to phenotypic abnormalities segregating within families or occurring sporadically are evaluated. Chromosomal findings are also correlated with genetic markers such as HLA and alpha-1-antitrypsin. Previous studies of the Fragile X chromosome and Alzheimer disease are being concluded, and analysis of variation in the Nucleolus Organizing Region in Down syndrome families is in progress. A limited number of patients with genetic syndromes such as Prader-Willi, familial aniridia, and fragile X were studied as part of our participation in the NIH genetics program.

Methods utilized include routine peripheral blood and tissue culturing, analysis of chromosomal response to folate-thymidine deprivation (fragile site induction), in-situ silver staining for detection of ribosomal gene activity of nucleolus organizing regions, chromosomal banding with Giemsa-trypsin or fluorescent stains, and methotrexate synchronization for analysis of prometaphase banding patterns.

Screening of proband's relatives is carried out to determine if carrier status of chromosomal variations is causally related to phenotypic abnormalities. Results of all studies are correlated with clinical information and utilized for purposes of genetic counseling (predicting risk for development or recurrence of disease within families).

Formerly Z01 AM 21,009-18 LCBG

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 21010-10 LCBG

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Function of DNA virus genomes in animal cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: Barrie J. Carter Chief, Section on Macromolecular Genetics LCBG:NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Cell Biology and Genetics

SECTION

Macromolecular Genetics

INSTITUTE AND LOCATION

NIDDK:NIH, Bethesda, Md. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has transferred to the Laboratory of Molecular and Cellular Biology.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 AM 21012-09 LCBG

PERIOD COVERED
October 1 through 31, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cytogenetics and Immunogenetics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: E.S. Raveche Research Chemist LCBG,NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Cell Biology and Genetics

SECTION
Cytogenetics

INSTITUTE AND LOCATION
NIH, NIDDK, Bethesda, Md. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 AM 21018-04 LCBG

PERIOD COVERED
October 1, 1985 to January 31, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies of membranes in macrophages and in bacteria

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	Loretta Leive	Chief, Section on Membrane Biology	LCBG:NIDDK
Others:	Victor Jiminez	Visiting Fellow	LCBG:NIDDK
	Gretchen Schieber	Staff Fellow	LCBG:NIDDK
	Emilie Klima	Chemist	LCBG:NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Cell Biology and Genetics

SECTION
Membrane Biology

INSTITUTE AND LOCATION
NIDDK:NIH, Bethesda, Md. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.06	.2	.04

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 21019-04 LCBG

(formerly Z01 AM 21019-03 LCGB)

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanism of hormone and transmitter secretion

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: Harvey B. Pollard Chief, Laboratory of Cell Biology and Genetics, NIDDK

Others: R. Ornberg, Ph.D., SSF; G. Lee, Ph.D., Res. Chemist; E. Rojas, Ph.D., VS; I. Atwater, Ph.D., Expert; R. Santos, VA; M. Levine, M.D., RA; K. Brocklehurst, Ph.D., VF; P. Lelkes, Ph.D., VS; L. Rosario, Ph.D., VF; E. Forsberg, Ph.D., SF; A. Burns, Ph.D., Expert; I. Cabantchik, Ph.D., GW; A. Stutzin, Ph.D., VF; V. N-Gentina Ph.D., GW; I.M. Perez, Ph.D., VF; M. Srivastava, Ph.D., GW; C. McCutchen, Ph.D., Res. Phys; M. Isosaki, Ph.D., VF; G. Kypers, Ph.D., VF; C. Artelejo, M.D., GW; G. Swergold, M.D., PRAT; K. Furuya, Ph.D., GW; A. Ramu, M.D., GW; N.P. Ramu, M.D., GW, S. Joost, M.D., VF; M. Li, M.D., VF; Y-Shi, Ph.D., GW; G. Goping, EM Tech.

COOPERATING UNITS *(if any)*

Dipak Banerjee, Ph.D., NIDR; David Rodbard, M.D., NICHD.

LAB/BRANCH

Laboratory of Cell Biology and Genetics

SECTION

Cell Biology and Biochemistry

INSTITUTE AND LOCATION

NIDDK:NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
23.5	29	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our recent studies have focussed on the processes underlying granule assembly, synthesis and insertion of hormones into granules, movement of granules to sites of secretion and signals leading to membrane contact and fusion between granules and plasma membranes leading to exocytosis in chromaffin cells and Islets of Langerhans, our primary experimental systems. In the chromaffin cell a key element in assembly is the enzyme cytochrome b562, since it is the only "intrinsic membrane protein." We have cloned this protein and are now determining its primary structure. The function of this cytochrome may be to donate electrons to the intragranular enzyme DBH, so that it can convert dopamine to norepinephrine. The origin of the electrons is ascorbic acid. Movement to the site of membrane fusion may be regulated by actin filaments, since dissolution of these filaments potentiates secretion. Secretion signals may include protein kinase C substrates and IP3, as well as calcium. We have purified protein kinase C to homogeneity and are studying its properties. The final event, membrane fusion, may be mediated by synexin, the receptors for which include acidic phospholipids and perhaps specific proteins. Protons may also facilitate synexin action. Secretion signals seem to originate on the plasma membrane, either due to binding to specific receptor or changes in electrical potential. In chromaffin cells nicotinic and muscarinic cholinergic receptors allow calcium to enter the cytosol, from either outside or inside the cell, respectively. In the B cell similar muscarinic receptors mediate secretion, but the site of glucose action remains unresolved. However, both types of secretagogues cause cyclic changes in activity of K^+ and Ca^{++} channels on the plasma membrane, resulting in cyclic changes in the rate of insulin secretion over time. Cyclic nucleotides also regulate coupling and secretory efficiency of B cells. The cytosolic pH may also regulate ionic channel function and secretion, but the latter two processes are not absolutely coupled.

PHS 6040 (Rev 1/84) GPO 904-917

ANNUAL REPORT OF THE LABORATORY OF BIOCHEMICAL PHARMACOLOGY
NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

I. POLYAMINES

Polyamine Biosynthesis in Escherichia coli: S-Adenosylmethionine Decarboxylase (speD) and Spermidine Synthase (speE). We have continued our studies of the genetics and regulation of the biosynthesis of spermidine in E. coli, emphasizing the two enzymes involved, S-adenosylmethionine decarboxylase and spermidine synthase. We are particularly interested in S-adenosylmethionine decarboxylase, both because it is essential for spermidine synthesis and because it requires covalently-linked pyruvate for activity. We are also very interested in spermidine synthase, since no previous information was available on the genetics of this enzyme.

In the current studies we have shown that the gene for spermidine synthase (speE) lies immediately adjacent to and upstream to the gene for S-adenosylmethionine decarboxylase (speD). Both genes are controlled by a single promoter upstream to the speE gene. Thus, these two genes form an operon and the genetic regulation of the speD gene is intimately dependent on the speE gene and its promoter.

We have purified both proteins to homogeneity, and Dr. Darrell T. Liu (DBB, CDB) has carried out partial protein sequencing. We have also sequenced the DNA coding for these enzymes and now have shown, by the identity of the amino acid sequences deduced from the DNA sequences with those of the protein fragments, that each gene codes for the respective structural protein.

We have shown that S-adenosylmethionine decarboxylase is formed as a proenzyme, which is then cleaved to produce two smaller fragments. We have identified the specific peptide bond that is cleaved to form the pyruvoyl group as a lysylserine linkage.

. . . . . Drs. H. Tabor, C. W. Tabor, and Q.-W. Xie

Polyamine Biosynthesis, Regulation, and Function in Saccharomyces cerevisiae. We have continued our studies of polyamines in Saccharomyces cerevisiae, utilizing biochemical and genetic methods.

We have emphasized the study of the gene for S-adenosylmethionine decarboxylase, an essential enzyme in polyamine biosynthesis, in S. cerevisiae. This protein is also important since we had shown that it has an unusual requirement for activity, namely, a covalently-linked pyruvate. We have prepared a 3200-bp fragment of yeast DNA that can be maintained stably in a shuttle yeast vector. This fragment of DNA contains the complete coding sequence for the enzyme and is expressed in yeast. We have evidence for the synthesis of a longer proenzyme that is cleaved to form an active pyruvate-containing subunit. We are sequencing the gene using the dideoxy technique in order to study the mechanism

of formation of the essential pyruvate that is covalently linked to the protein.

We have shown that a cell division cycle defect occurs in amine-deficient cells, with the accumulation of budded forms. The more complete the amine deficiency, the more nearly equal the size of the mother cell and the bud. Thus we have shown that polyamine deficiency is associated with arrest of the cycle in a late period, just before cytokinesis.

Ornithine decarboxylase, the enzyme that synthesizes putrescine in S. cerevisiae, has now been purified (75% pure) as an 86,000 M_r polypeptide under conditions that minimize proteolysis. We find that the enzyme is synthesized as a 86,000 M_r polypeptide that is easily and specifically converted to the 68,000 M_r form by proteolysis.

. . . . . Drs. C. W. Tabor, H. Tabor, and S. K. Taneja

II. MECHANISMS OF INHERITANCE IN SACCHAROMYCES CEREVISIAE

Yeast has 5 families of double-stranded RNAs (dsRNAs) replicating in its cytoplasm. These are called L-A, L-BC, M, T, and W. L-A, L-BC, and M are found in intracellular, noninfectious virus-like particles (VLPs). M encodes a secreted toxin and immunity to that toxin, while L-A encodes the major protein of the VLPs in which both itself and M are encapsidated. We have established an *in vitro* replication system for L-A, L-BC, and M. We find that (-) strand L-A synthesis occurs in VLPs carrying only the (+) strand, while (+) strand synthesis occurs by a conservative mechanism in particles carrying L-A dsRNA. New (+) strands are extruded from the VLPs. M replication occurs *in vitro* by a mechanism similar to that of L-A except that, because M is less than half the size of L-A, the same particles that can only hold one L-A can hold one or two M molecules. [D], a new cytoplasmic genetic element we discovered, exacerbates the disease produced by the derepression of M dsRNA replication seen in *ski*⁻ mutants. We have isolated chromosomal mutants unable to maintain [D] (*mad*⁻). The *MAK* genes are chromosomal genes essential for M dsRNA replication, while the *SKI* genes are repressors of this process. We have isolated clones of *MKT1*, *MAK11*, *MAK16*, *MAK18*, *SKI3*, *SKI8*, and *CDC16*. We have completely sequenced the *MAK11*, *MAK16*, and *CDC16* genes.

. . . . . Drs. R. B. Wickner, T. Fujimura, R. Esteban, T. Icho, and H.-S. Lee

III. NUCLEIC ACIDS

All mammals contain several families of repetitive DNA sequences that comprise at least one-third of the genome. We have continued our characterization of the rat long, interspersed, repeated DNA family (or LINE family). This included determination of the DNA sequence of a full length (6.7 kb) member and of parts of several other members. Comparisons among these sequences and restriction enzyme analysis of the 40,000

or so genomic copies of the family showed that the sequenced 6.7 kb member is very typical of the family, both with respect to length and overall structure. Therefore, the rat LINE family is quite homogeneous, which is in marked contrast to the primate and mouse LINE families which are quite heterogeneous, and accounts for about 10% of the rat genome. Rat LINE members are highly transcribed, and their transcripts account for a substantial part of the nuclear RNA of various normal rat cells. Rat LINE members contain numerous long open reading frames, and potential regulatory sequences are present at both termini. Furthermore, the regulatory sequences in one of the termini strongly arrest DNA synthesis in vitro. This result is quite gratifying, since the presence of DNA arrest sites in chromosomal DNA has been surmised, but never demonstrated, and arrest sites have been implicated in some mechanisms for the amplification and transposition of mammalian DNA sequences. By using deleted and base substituted versions of these arrest site sequences, we have established the biochemical requirements for DNA arrest in vitro and are now examining the properties of these sites in vivo. DNA sequence analysis showed that several chromosomal target sites at which full length members have inserted, although not homologous overall, share a structural motif that clearly defines a class of target sites. Since LINE insertion is due to an illegitimate recombinational event and since such events underly many important normal and pathological genetic rearrangements in mammalian genomes, these results are quite provocative, especially in light of the paucity of information on what governs illegitimate recombination.

. . . . . Drs. A. V. Furano, F. T. Robb, E. D'Ambrosio, I. Nur, K. Usdin, and S. M. Robb, and A. Salemme; Dr. P. Tsichlis (Fox Chase Cancer Center, Philadelphia)

DNA Replication: The Bacteriophage T4 System. We are using bacteriophage T4 as a model system for studying the complex enzymatic reactions needed for duplex DNA replication. Replication requires a minimum of seven phage encoded proteins: gene 43 T4 DNA polymerase, gene 32 DNA helix-destabiling protein, genes 44/62 and 45 polymerase accessory proteins, and the gene 41 and 61 primase-helicase proteins.

Primase-Helicase Complex. 61 protein alone has a limited primer synthesis activity, while 41 protein alone has a DNA unwinding (helicase) activity. The two proteins together form a functional complex that catalyzes both activities much more efficiently than the individual proteins. Although 61 protein alone (at very high concentrations) makes mainly the dimers pppAC and pppGC, only the rare oligonucleotides which begin with GC and are long enough to stably hybridize to the DNA template are able to initiate new DNA chains in the absence of 41 protein. In contrast, with catalytic concentrations of 41 and 61 proteins together, new chains begin with the pentamers $pppACN_3$ or $pppGCN_3$, except with T4 DNA (containing hydroxylmethyl cytosine in place of cytosine), where there are no chains beginning with G. These results suggest that 41 protein changes the predominant oligonucleotide made from dimers to pentamers, limits the synthesis of oligomers > 5, and may be necessary to keep the pentamers bound to the template so that they can be

elongated by T4 DNA polymerase. Moreover, since 61 protein alone only initiates DNA chains beginning with GC, but is unable to make GC oligonucleotides on T4 DNA, both the T4 61 and 41 proteins are essential for discontinuous synthesis on their normal DNA template.

We have proposed and are currently testing a model in which the physical interaction of the polymerase-accessory protein complex with the primase-helicase controls the selection of priming sites for the initiation of new fragments on the lagging strand. For this purpose we have constructed forked circular duplex DNA molecules of defined sequence. We have shown that the T4 replication proteins assemble more rapidly and synchronously on these templates than on comparable nicked circles, and will now use these forked molecules to study what factors regulate which of the potential template sequences for primer synthesis are actually used to initiate new DNA chains.

We have constructed plasmids expressing high levels of 41 protein with N-terminal and C-terminal deletions. We have purified these altered proteins to determine if they retain domains of 41 protein needed for its primase stimulation, helicase, or DNA-dependent nucleotidase activities.

We are collaborating with Dr. David Ollis (Northwestern University) to obtain crystals of the 41 and 61 proteins suitable for x-ray analysis.

. . . . . Drs. D. M. Hinton, R. W. Richardson, and N. G. Nossal

Expression of T4 DNA Replication Proteins. Most T4 DNA replication proteins are made in the early and middle stages of infection. We have previously cloned a region of the T4 genome, including genes for the uvsX recombination protein, the 41 and 61 priming proteins, and the DNA adenine methylase (dam) protein. We are now using these clones to study how the expression of these genes is controlled. Preliminary evidence from this and other laboratories suggests that early T4 genes have promoter sequences that can be recognized by the normal host E. coli RNA polymerase, and that expression of the middle and late gene products is then controlled by several different T4 proteins which allow recognition of other promoter sequences by the host polymerase or prevent transcription termination.

We have located two strong promoters 700-800 bp upstream of uvsX which are recognized by the unmodified E. coli RNA polymerase. Using expression vectors developed by McKenny and Rosenberg, we find that plasmids containing the uvsX promoters express 30- to 60-fold more galactokinase than control plasmids, which is high enough to cause the cells to grow poorly. This sick phenotype is reversed by cloning the strong IS2 transcription termination sequence between the T4 DNA and the galK gene. In addition, healthy revertants arise spontaneously at low frequency by insertion of the E. coli transposable elements IS1 or IS5 downstream of the uvsX promoters. These transposons have been shown by others to block transcription from downstream genes. Preliminary evidence suggests that the higher level of expresion of the uvsX relative to the 41

protein results at least in part from a transcription terminator located between the two genes.

. . . . . Dr. D. M. Hinton

T4 DNA Adenine Methylase. The T4 adenine methylase (dam) methylates the A in the sequence GATC. We find that expression of the T4 dam gene in pBR322-based plasmids causes E. coli cells to grow poorly, particularly if they are defective in RNase H. We will test whether this growth inhibition results from methylation of the GATC sequences present in the DNA replication origins of both the E. coli chromosome and pBR322 plasmids.

. . . . . Dr. N. G. Nossal and Dr. R. J. Crouch (LMG, NICHD)

Hepatitis Non-A, NonB. Hepatitis non-A, nonB (HNANB) is a world-wide problem, and 90% of the transfusion-related hepatitis cases in the United States (and 80-90% in several other countries) are diagnosed as HNANB. Approximately 50% of all acute HNANB patients develop chronic HNANB (an estimate of 4 million persons). They remain as potential sources of infection. Recent publications suggest a correlation between certain hepatocellular carinomas and chronic HNANB infections.

Based on biochemical, immunlogical, and morphological evidence, we suggested that the HNANB agent is a mammalian type C retrovirus. Recently, using an in vitro focus-induction assay developed for mammalian type C viruses, we observed that pelleted material from HNANB sera (transfusion-related) induced foci formation. This result is consistent with the presence of a mammalian type C virus in HNANB sera.

. . . . . Drs. W. G. Coleman, Jr., and B. P. Seto (DBBP, NCDB, FDA)

IV. MEMBRANE STUDIES OF MACROPHAGES AND OF ESCHERICHIA COLI

Aldoheptose Biosynthesis. Previously, a novobiocin-hypersensitive mutant of Escherichia coli K-12 carrying a cysE-pyrE linked mutation, designated rfaD, which specifically affects the synthesis of the aldoheptose, L-glycero-D-mannoheptose, has been isolated and genetically characterized. Recently, we have cloned a 2.5 kilobase fragment carrying the rfaD gene into several high copy plasmids. The rfaD gene has been expressed in several expression systems, and further characterization of the rfaD gene product is in progress.

. . . . . Drs. J. C. Pegues and W. G. Coleman, Jr.

V. ENZYME MECHANISMS AND PROTEIN STRUCTURE

We have prepared crystals of the Salmonella typhimurium tryptophan synthase $\alpha_2\beta_2$ complex which are suitable for structural analysis by x-ray crystallography. Several complete data sets have been collected at 2.8 Å on the native crystals and on several heavy metal derivatives. Studies of the activity of the crystals are being made with

microcrystals and also by microspectrophotometry of single crystals in the presence and absence of substrates.

. . . . . Drs. E. W. Miles and S. A. Ahmed; D. R. Davies and C. C. Hyde (LMB, NIDDK); A. Mozarelli (University of Parma, Italy)

We are engineering specific amino acid changes in tryptophan synthase by oligonucleotide-dependent site-specific mutagenesis. Four mutants in the α subunit have been prepared: Arg-179 → Leu, Cys-81 → Ser, Cys-118 → Ser, and Cys-154 → Ser. A series of 5 active site mutants in the β_2 subunit are in progress. The Arg-179 mutant has been purified, crystallized, and is being characterized. The site of mutation appears to be important for the transmission of a substrate-induced conformational change from the α subunit to the β_2 subunit. The several mutants substituted at cysteines have been designed to help in solving the x-ray structure of mercury derivatives by removing specific mercury binding sites. When the x-ray structure is solved, the structure should identify key active site residues which can be further studied by making mutants at these positions. We are also collaborating with K. Yutani on a series of 20 mutants at Glu-49 in the α subunit.

. . . . . Drs. E. W. Miles, H. Kawasaki, and S. A. Ahmed; G. Zon (CDB, DBB); R. Bauerle (University of Virginia); K. Yutani (University of Osaka, Japan)

Comparative studies of tryptophan synthase and tryptophanase with a reaction intermediate analog, 2,3-dihydro-L-tryptophan, show that the two enzymes differ in the stereochemical course of intermediate formation although they share similar reaction mechanisms. This basic similarity in reaction mechanism is supported by our recent finding that tryptophan synthase slowly cleaves L-tryptophan, a reaction previously thought to be catalyzed only by tryptophanase. Further studies with analogs of D- and L-tryptophan and their 5-fluoro derivatives using ^{19}F-NMR and difference spectroscopy have led to the discovery of new isomerization reactions catalyzed by tryptophan synthase. These studies further define the stereochemistry and mechanism of this enzyme.

. . . . . Drs. E. W. Miles and S. A. Ahmed; R. S. Phillips, L. C. Cohen, and H. J. C. Yeh (LC, NIDDK)

The β_2 dimer of tryptophan synthase has proved to be a useful enzyme for studying pressure dissociation and conformational drift. The pressure-induced dissociation of the dimer to monomer and reassociation have been followed by measuring the intrinsic fluorescence and the fluorescence of the pyridoxal phosphate chromophore as well as changes in activity.

. . . . . Drs. E. W. Miles; J. L. Silva and G. Weber (University of Illinois)

The hinge region connecting the two domains of the β_2 subunit of tryptophan synthase is being probed by studies using limited proteolysis. Three sites of cleavage have been identified: Arg-275, Lys-272, and

Lys-283. This hinge region is important for activity and subunit interaction.

. . . . . Drs. E. W. Miles and S. A. Ahmed; T. Fairwell (MD, NHLBI); K. Kirschner (University of Basel, Switzerland)

A method for accurately measuring the amount of each of two differently radiolabeled chemical species in a mixture has been developed. This method has been utilized, in conjunction with an automated microfractionator previously developed in this laboratory, to quantitate the concentration gradients, formed under the influence of centrifugal force fields, of each of two solute species in a mixture.

. . . . . Drs. A. K. Attri and A. P. Minton

A theory of sedimentation equilibrium has been extended to solutions of one or two solute components, which may undergo self- or hetero-association, at arbitrarily high concentrations. Simple yet accurate semi-empirical algorithms have been developed to calculate the true weight-average molecular weight of each component from the experimentally measured apparent weight-average molecular weight.

. . . . . Drs. R. C. Chatelier and A. P. Minton

The kinetics of formation of concentration gradients in a solution subjected to a time-varying centrifugal force are being studied by means of computer simulation.

. . . . . Dr. A. P. Minton

A complex valyl-tRNA synthetase has been found to undergo an oscillatory interconversion between two forms that are separable in 50% ammonium sulfate. The process is affected by oxidants such as the pyridine nucleotides and reductants such as thiols, and is suggested to be part of a regulatory mechanism.

. . . . . Dr. S. Black

Cooperative binding systems are being studied taking into account site or subunit interactions, ligand interactions, aggregation and redistribution in proteins, and model systems. Methods are being developed to evaluate reasonable values for the parameters describing these systems.

Amino acid sequences of proteins are analyzed primarily with the Monte Carlo techniques to evaluate the uniqueness and homology of these sequences. The property of uniqueness (the occurrence of a small peptide at a frequency considerably less than that expected) has been quantified, and speculations on this quantity and the immune response have been presented.

. . . . . Drs. H. A. Saroff and E. Mihalyi

Methods of molecular weight estimation require purified samples for detection of individual structures. An alternative approach utilizes the random damage to molecules by ionizing radiation. Under appropriate experimental conditions, this damage leads to complete loss of function which can be described by target theory. Measurement of surviving function after exposure of frozen samples to high energy electrons permits calculation of the target size, which is interpreted as the mass of all the molecular structures which are required for the measured activity. The radiation technique therefore gives an estimate of the size of the functionally-active structure. The conditions under which this approach is valid requires that the radiation damage be directly on the active unit. Damage to other components are without effect on the measurement. It then follows that impure samples can be used, even intact cells and tissues. Not only does this enable estimates of molecular weights in vivo, it also allows the simultaneous determination of a large number of unrelated molecular weights from the same experimental sample.

. . . . . Dr. E. S. Kempner

VI. DYNAMIC PROPERTIES OF CELL MEMBRANES AND RELATED SYSTEMS

The objectives of this project are to understand the molecular mechanisms utilized by cells for regulating the lipid composition of their membranes, and to establish the processes that lead to the assembly of the lipid bilayer. Two current problems associated with these goals are why the lipid composition of membranes changes when growth temperatures are altered, and why membrane bilayers are unilamellar. Our studies with aqueous phospholipid dispersions indicate that the unilamellar (single bilayer) state forms spontaneously, but only at a unique temperature that depends on the composition of the phospholipid in the dispersion. The temperature at which the unilamellar state forms is a critical point T*; above and below this temperature, other lipid states form which are not unilamellar. The lipid composition requirements for the formation of the unilamellar state have been identified for a number of synthetic phospholipid mixtures, and for a series of cell membrane lipid extracts. In each case the critical temperature T* is characteristic of the lipid composition. Moreover, in the case of the membrane lipid extracts, T* was the same as the growth temperature of the cell from which the lipids were extracted. Since T* represents the temperature where only the single bilayer state forms, the agreement between T* and cell growth temperatures suggests that membrane bilayer assembly in cells may occur by a spontaneous process similar to that observed in the lipid dispersions. This assembly mechanism also accounts for the composition changes encountered when growth temperatures are altered. Since the single bilayer state forms with a characteristic composition at T*, any change in the growth temperature would require a corresponding change in lipid composition if new membrane bilayer is to form. A rigorous test of this hypothesis is currently in progress.

. . . . . Dr. N. L. Gershfeld

VII. CULTURE OF MARROW CELLS AND OF MYCOBACTERIUM

During my cinemicrographic studies on long-term growth of bone marrow cells, a new type of net-like stromal cell appeared in the culture. The cell was very large, measuring more than 500 micrometer in diameter. The cytoplasm was very thin, but enforced with cytoplasmic strands arranged like a fishing net. Frequent appearance and disappearance of small and large holes in the cytoplasm was observed. There were two nuclei, each containing a prominent nucleolus. The cell remained more or less stationary throughout a period of 44-hour continued cinemicrography and was still in good condition at the end of observation. These features suggested that this new type of cell might be the mysterious, not yet cultivated reticulum cell of the bone marrow. The cell was observed in a liquid culture system developed in this laboratory. Good growth of macrophages, granulocytes, and megakaryocytes was also observed. Short motion pictures of these cells are available.

. . . . . Dr. Y. T. Chang

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 23,140-28 LBP

(formerly Z01 AM 23,140-27 LBP)

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biochemistry of Sulfur-Containing Compounds

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Simon Black, Ph.D. Biochemist and Assistant Chief, LBP LBP NIDDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Biochemical Pharmacology

SECTION

Section on Pharmacology

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	1.0	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A complex valyl-tRNA synthetase has been found to undergo an oscillatory interconversion between two forms that are separable in 50% ammonium sulfate. The process is affected by oxidants such as the pyridine nucleotides and reductants such as thiols, and is suggested to be part of a regulatory mechanism.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 23,230-36 LBP

(formerly Z01 AM 23,230-35 LBP)

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Chemotherapy of Mouse Leprosy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Yao Teh Chang, M.D. Research Pharmacologist LBP NIDDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Biochemical Pharmacology

SECTION

Section on Pharmacology

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	1.0	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 23,330-08 LBP

(formerly Z01 AM 23,330-07 LBP)

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Aldoheptose Biosynthesis and Its Regulation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	William G. Coleman, Jr., Ph.D.	Research Microbiologist	LBP NIDDK
Others:	Joyce C. Pegues, Ph.D.	Staff Fellow	LBP NIDDK
	Jewell D. Wilson, Ph.D.	Staff Fellow	LBP NIDDK

COOPERATING UNITS *(if any)*

Belinda P. Seto, Ph.D., Research Chemist, DBBP, NCDB, FDA

LAB/BRANCH

Laboratory of Biochemical Pharmacology

SECTION

Section on Pharmacology

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.1	2.8	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Aldoheptose Biosynthesis. Previously, a novobiocin-hypersensitive mutant of Escherichia coli K-12 carrying a cysE-pyrE linked mutation, designated rfaD, which specifically affects the synthesis of the aldoheptose, L-glycero-D-mannoheptose, has been isolated and genetically characterized. Recently, we have cloned a 2.5 kilobase fragment carrying the rfaD gene into several high copy plasmids. The rfaD gene has been expressed in several expression systems, and further characterization of the rfaD gene product is in progress.

Hepatitis Non-A, NonB. Hepatitis non-A, nonB (HNANB) is a world-wide problem, and 90% of the transfusion-related hepatitis cases in the United States (and 80-90% in several other countries) are diagnosed as HNANB. Approximately 50% of all acute HNANB patients develop chronic HNANB (an estimate of 4 million persons). They remain as potential sources of infection. Recent publications suggest a correlation between certain hepatocellular carinomas and chronic HNANB infections.

Based on biochemical, immunlogical, and morphological evidence, we suggested that the HNANB agent is a mammalian type C retrovirus. Recently, using an in vitro focus-induction assay developed for mammalian type C viruses, we observed that pelleted material from HNANB sera (transfusion-related) induced foci formation. This result is consistent with the presence of a mammalian type C virus in HNANB sera.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 23,580-23 LBP

(formerly Z01 AM 23,580-22 LBP)

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Gene Expression in the Rat and Other Organisms

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Anthony V. Furano, M.D.	Medical Officer (Research) and Chief, Section on Genomic Structure and Function, LBP	LBP NIDDK
Others:	Frank T. Robb, Ph.D.	Visiting Scientist	LBP NIDDK
	Ettore D'Ambrosio, Ph.D.	Visiting Fellow	LBP NIDDK
	Israel Nur, Ph.D.	Visiting Fellow	LBP NIDDK
	Karen Usdin, Ph.D.	Visiting Fellow	LBP NIDDK
	Susan M. Robb, Ph.D.	Guest Researcher	LBP NIDDK
	Anne Salemme, B.A.	Guest Researcher	LBP NIDDK

COOPERATING UNITS *(if any)*

Dr. Philip Tsichlis, Fox Chase Cancer Center, Philadelphia, Pennsylvania

LAB/BRANCH

Laboratory of Biochemical Pharmacology

SECTION

Section on Genomic Structure and Function

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.2	4.0	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

All mammals contain several families of repetitive DNA sequences that comprise at least one-third of the genome. We have continued our characterization of the rat long, interspersed, repeated DNA family (or LINE family). This included determination of the DNA sequence of a full length (6.7 kb) member and of parts of several other members. Comparisons among these sequences and restriction enzyme analysis of the 40,000 or so genomic copies of the family showed that the sequenced 6.7 kb member is very typical of the family, both with respect to length and overall structure. Therefore, the rat LINE family is quite homogeneous, which is in marked contrast to the primate and mouse LINE families which are quite heterogeneous, and accounts for about 10% of the rat genome. Rat LINE members are highly transcribed, and their transcripts account for a substantial part of the nuclear RNA of various normal rat cells. Rat LINE members contain numerous long open reading frames, and potential regulatory sequences are present at both termini. Furthermore, the regulatory sequences in one of the termini strongly arrest DNA synthesis in vitro. This result is quite gratifying, since the presence of DNA arrest sites in chromosomal DNA has been surmised, but never demonstrated, and arrest sites have been implicated in some mechanisms for the amplification and transposition of mammalian DNA sequences. By using deleted and base substituted versions of these arrest site sequences, we have established the biochemical requirements for DNA arrest in vitro and are now examining the properties of these sites in vivo. DNA sequence analysis showed that several chromosomal target sites at which full length members have inserted, although not homologous overall, share a structural motif that clearly defines a class of target sites. Since LINE insertion is due to an illegitimate recombinational event and since such events underly many important normal and pathological genetic rearrangements in mammalian genomes, these results are quite provocative, especially in light of the paucity of information on what governs illegitimate recombination.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT (formerly

PROJECT NUMBER

ZO1 DK 23,600-17 LBP
ZO1 AM 27,003-16 LPB)

PERIOD COVERED
October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Dynamic Properties of Cell Membranes and Related Systems

PRINCIPAL INVESTIGATOR *(List other professional personnal below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Norman L. Gershfeld, Ph.D. Research Chemist LBP NIDDK

COOPERATING UNITS *(if any)*

Dr. Ralph J. Nossal, PSL, DCRT, and Dr. Robert L. Berger, LTD, NHLBI

LAB/BRANCH
Laboratory of Biochemical Pharmacology

SECTION
Section on Physical Biology

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objectives of this project are to understand the molecular mechanisms utilized by cells for regulating the lipid composition of their membranes, and to establish the processes that lead to the assembly of the lipid bilayer. Two current problems associated with these goals are why the lipid composition of membranes changes when growth temperatures are altered, and why membrane bilayers are unilamellar. Our studies with aqueous phospholipid dispersions indicate that the unilamellar (single bilayer) state forms spontaneously, but only at a unique temperature that depends on the composition of the phospholipid in the dispersion. The temperature at which the unilamellar state forms is a critical point T*; above and below this temperature, other lipid states form which are not unilamellar. The lipid composition requirements for the formation of the unilamellar state have been identified for a number of synthetic phospholipid mixtures, and for a series of cell membrane lipid extracts. In each case the critical temperature T* is characteristic of the lipid composition. Moreover, in the case of the membrane lipid extracts, T* was the same as the growth temperature of the cell from which the lipids were extracted. Since T* represents the temperature where only the single bilayer state forms, the agreement between T* and cell growth temperatures suggests that membrane bilayer assembly in cells may occur by a spontaneous process similar to that observed in the lipid dispersions. This assembly mechanism also accounts for the composition changes encountered when growth temperatures are altered. Since the single bilayer state forms with a characteristic composition at T*, any change in the growth temperature would require a corresponding change in lipid composition if new membrane bilayer is to form. A rigorous test of this hypothesis is currently in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 23,630-20 LBP

PERIOD COVERED

September 30, 1985

TITLE OF PROJECT *(80 characters or lass. Title must fit on one line between the borders.)*

Biology of Complex Carbohydrates

PRINCIPAL INVESTIGATOR *(List other professional personnel below tha Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Victor Ginsburg, Ph.D. Research Chemist and Chief, Section on Biochemistry, LBP LBP NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Biochemical Pharmacology

SECTION

Section on Biochemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use stenderd unreduced type. Do not exceed the space provided.)*

This project was transferred to the Section on Biochemistry, Laboratory of Structural Biology, NIDDK. The new project number for the period October 1, 1985 to September 30, 1986 is Z01 DK 57,000-21 LSB. Please see Project Number Z01 DK 57,000-21 LSB for Summary of Work and Project Description.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 AM 23,830-06 LBP

PERIOD COVERED
September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Electrochemical Ion Gradients as a Mechanism of Cellular Message Transmission

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Evelyn F. Grollman, M.D. Medical Officer (Research) LBP NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Biochemical Pharmacology

SECTION
Section on Biochemistry of Cell Regulation

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project was transferred to the Section on Cell Regulation, Laboratory of Biochemistry and Metabolism, NIDDK. The new project number for the period October 1, 1985 to September 30, 1986 is Z01 DK 18,007-01 LBM. Please see Project Number Z01 DK 18,007-01 LBM for Summary of Work and Project Description.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 23,860-27 LBP

(formerly Z01 AM 27,008-26 LPB)

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biophysical Studies of Metabolic Activity and Control

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Ellis S. Kempner, Ph.D. Physicist, and Chief, Section on Physical Biology LBP NIDDK

COOPERATING UNITS *(if any)*

Drs. M. J. McCreery (Letterman Army Institute of Research); S. Pestka (Roche Institute); R. Wood (University of Georgia); R. Salovey (University of Southern California)

LAB/BRANCH

Laboratory of Biochemical Pharmacology

SECTION

Section on Physical Biology

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standerd unreduced type. Do not exceed the space provided.)*

Molecular weight of specific biopolymers is an important parameter in characterizing the molecule, its position and orientation in situ, and its interactions with other compounds. Measurements of molecular weight are accomplished by several different techniques which depend on chemical or physical properties of these structures, and all of which demand purified samples. An alternative method is that of radiation inactivation. By means of target analysis it is possible to evaluate a molecular weight which depends on different properties, the functional activities of the specific biopolymer. It is an important feature of this technique that crude samples can be used.

The physical basis for this approach has been described long ago, but as the method is applied to more complicated systems and more complex structures, new questions arise. These involve interactions between subunits of proteins, effects of a lipid environment as in membranes, or the presence of carbohydrates. Experimental procedures which modify the natural order of things -- treatment with detergents, for example -- could also change the radiation-sensitive unit detected by enzymatic activity or receptor function. Analysis of these effects is an important phase of this project on molecular weight determination by radiation inactivation.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 23,960-19 LBP

PERIOD COVERED

September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cell Regulation by the Action of Pharmacodynamic Agents on the Cell Membrane

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Leonard D. Kohn, M.D. Medical Director, USPHS, and
Chief, Section on Biochemistry of Cell Regulation, LBP LBP NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Biochemical Pharmacology

SECTION

Section on Biochemistry of Cell Regulation

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project was transferred to the Section on Cell Regulation, Laboratory of Biochemistry and Metabolism, NIDDK. The new project number for the period October 1, 1985 to September 30, 1986 is Z01 DK 18,008-01 LBM. Please see Project Number Z01 DK 18,008-01 LBM for Summary of Work and Project Description.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 24,140-20 LBP

(formerly Z01 AM 24,140-19 LBP)

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Tryptophan Synthase: Structure and Function and Relationship to Tryptophanase

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Edith Wilson Miles, Ph.D.	Research Chemist	LBP NIDDK
Others:	Syed A. Ahmed, Ph.D.	Visiting Fellow	LBP NIDDK
	Haruhiko Kawasaki, Ph.D.	Visiting Fellow	LBP NIDDK

COOPERATING UNITS *(if any)* Drs.T.Fairwell,MD,NHLBI; D.Davies,C.Hyde,LMB,NIDDK; B.Martin, DMN,NINCDS; R.Phillips,L.Cohen,H.Yeh,LC,NIDDK; R.Bauerle,Biol.Dept.,Univ.Virginia, Charlottesville,VA; K.Kirschner,Biozentrum,Univ.of Basel,Switzerland; J.L.Silva, G.Weber,Univ.Illinois; A.Mozzarelli, Univ.Parma,Italy; K. Yutani,Osaka Univ.,Japan

LAB/BRANCH

Laboratory of Biochemical Pharmacology

SECTION

Section on Pharmacology

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.3	3.0	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are studying the structure and the reaction mechanism of the bacterial tryptophan synthase α2β2 complex. This multienzyme complex is the product of a well studied genetic system and is overproduced by some strains of bacteria. The chromophoric coenzyme, pyridoxal phosphate, serves as a reporter group of events at the active site of the β2 subunit and of changes in conformation of the β2 subunit. We are comparing the mechanism of tryptophan synthase with that of a closely related pyridoxal phosphate enzyme, tryptophanase. We find that these two enzymes share the same basic reaction mechanism but differ vastly in the rates of some of their individual reactions. They also differ in the steric course of formation of a reaction intermediate, the indolenine tautomer of L-tryptophan. Our studies of both enzymes have been facilitated by the use of a series of analogs of L- and D-tryptophan and of 5-fluoro-L- and D-tryptophan. Studies using 19F-NMR and difference spectroscopy have led to the discovery of two new isomerization reactions and of a cleavage reaction catalyzed by tryptophan synthase. These studies further define the stereochemistry and mechanism of tryptophan synthase. The tryptophan analogs are also useful in studies of the activity of crystals of the α2β2 complex of tryptophan synthase from Salmonella typhimurium. We have demonstrated that microcrystals of the enzyme are active. Single crystals studied by microspectrophotometry show distinctive spectral changes in the presence of substrates and analogs. We plan to use these analogs in x-ray crystallographic studies after the structure of the native crystal is solved. Data collected at 2.8 Å on the native crystal and on several metal derivatives is now being analyzed. We are engineering specific amino acid changes in tryptophan synthase by oligonucleotide-dependent site-specific mutagenesis. The first of these mutants in the α subunit (Arg 179 to Leu) has been expressed, purified, crystallized, and is being characterized. Other mutants in which a cysteine is replaced with serine have been designed to help in the solution of the x-ray structure by removing specific mercury binding sites.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 24,150-15 LBP

(formerly Z01 AM 24,150-14 LBP)

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Noncovalent Intermolecular Interactions in Biochemistry

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Allen P. Minton, Ph.D.	Research Chemist	LBP NIDDK
Others:	Ronald C. Chatelier, Ph.D.	Visiting Fellow	LBP NIDDK
	Nobuhiro Muramatsu, Ph.D.	Visiting Fellow	LBP NIDDK
	Arun K. Attri, Ph.D.	Guest Researcher	LBP NIDDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Biochemical Pharmacology

SECTION

Section on Pharmacology

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	3.3	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A method for accurately measuring the amount of each of two differently radio-labeled chemical species in a mixture has been developed. This method has been utilized, in conjunction with an automated microfractionator previously developed in this laboratory, to quantitate the concentration gradients, formed under the influence of centrifugal force fields, of each of two solute species in a mixture.

A theory of sedimentation equilibrium has been extended to solutions of one or two solute components, which may undergo self- or hetero-association, at arbitrarily high concentrations. Simple yet accurate semi-empirical algorithms have been developed to calculate the true weight-average molecular weight of each component from the experimentally measured apparent weight-average molecular weight.

The kinetics of formation of concentration gradients in a solution subjected to a time-varying centrifugal force are being studied by means of computer simulation.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT (formerly

PROJECT NUMBER

ZO1 DK 24,260-20 LBP
ZO1 AM 24,260-19 LBP)

PERIOD COVERED
October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Enzymatic Mechanisms of DNA Replication: The Bacteriophage T4 System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Nancy G. Nossal, Ph.D. Research Chemist and Chief, Section on Nucleic Acid Biochemistry, LBP LBP NIDDK

Others: Deborah M. Hinton, Ph.D. Senior Staff Fellow LBP NIDDK
Ross W. Richardson, Ph.D. Staff Fellow LBP NIDDK

COOPERATING UNITS *(if any)*
Dr. Robert Crouch, LMG, NICHD, and Dr. David Ollis, Department of Biochemistry, Northwestern University, Evanston, Illinois

LAB/BRANCH
Laboratory of Biochemical Pharmacology

SECTION
Section on Nucleic Acid Biochemistry

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	3.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are studying the E. coli bacteriophage T4 as a model system for duplex DNA replication. Efficient DNA replication in vitro is achieved with seven purified proteins encoded by T4 phage: T4 DNA polymerase (gene 43 product), gene 32 DNA helix-destabilizing protein, the gene 44/62 and gene 45 polymerase accessory proteins, and the genes 41 and 61 priming proteins.

61 protein alone has a limited primase activity and 41 protein alone has a DNA unwinding (helicase) activity. The two proteins together form a functional complex that catalyzes both activities much more efficiently than the individual proteins. Although 61 protein alone synthesizes mainly the dimers pppAC and pppGC, only the rare oligonucleotides which begin with G and are long enough to hybridize stably to the DNA template are able to initiate new DNA chains in the absence of 41 protein, in contrast to the pentanucleotide primers found at the ends of DNA with the 41 and 61 proteins together. On T4 DNA, which has hydroxymethyl cytosine in place of cytosine, DNA chain initiation requires both the 41 and 61 proteins. We have proposed and are currently testing a model in which the interaction of the polymerase-accessory protein complex with the primase-helicase controls the selection of priming sites for the initiation of new fragments on the lagging strand. We have constructed plasmids expressing 41 protein with N-terminal and C-terminal deletions and have purified these proteins to identify 41 protein domains required for its primase, helicase, and DNA-dependent nucleotidase activities.

We are studying the factors and sequences regulating the expression of the T4 DNA replication proteins, including genes uvsX (recombination protein) through genes 41, 61, and dam (DNA adenine methylase). We have located two strong transcription promoters 700-800 bp upstream of uvsX which are active in vivo and in vitro. A transcription terminator between genes uvsX and 41 contributes to the differential levels of these two proteins. The T4 dam protein methylates the A in the sequence GATC. Expression of the T4 dam gene in pBR322-based plasmids causes E. coli cells to grow poorly, particularly if they lack RNase H.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 24,590-15 LBP

(formerly Z01 AM 24,590-14 LBP)

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure and Interactions of Biologically Important Macromolecules

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Harry A. Saroff, Ph.D.	Scientist Emeritus and Special Expert	LBP NIDDK
	Elemer Mihalyi, M.D., Ph.D.	Guest Researcher	LBP NIDDK

COOPERATING UNITS *(if any)*

Clinical Endocrinology Branch, NIDDK, NIH, and National Center for Drugs and Biologics

LAB/BRANCH

Laboratory of Biochemical Pharmacology

SECTION

Section on Pharmacology

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.3	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Cooperative binding systems are being studied taking into account site or subunit interactions, ligand interactions, aggregation and redistribution in proteins, and model systems. Methods are being developed to evaluate reasonable values for the parameters describing these systems.

Amino acid sequences of proteins are analyzed primarily with the Monte Carlo techniques to evaluate the uniqueness and homology of these sequences. The property of uniqueness (the occurrence of a small peptide at a frequency considerably less than that expected) has been quantified, and speculations on this quantity and the immune response have been presented.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 AM 24,640-14 LBP

PERIOD COVERED
September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
(I) Polarography of Carcinogens and (II) Reactions of Formaldehyde

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigetor.) (Neme, title, leboretory, and institute affiliation)*

PI: Richard B. Simpson, Ph.D. Guest Researcher LBP NIDDK

COOPERATING UNITS *(if eny)*

None

LAB/BRANCH
Laboratory of Biochemical Pharmacology

SECTION
Section on Pharmacology

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the spece provided.)*

This project was terminated on September 30, 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 24,709-05 LBP

(formerly Z01 AM 24,709-04 LBP)

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)* (was Purification of Ornithine..)

Biochemical and Genetic Studies on Polyamine Biosynthesis in E. coli in Yeast

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Celia White Tabor, M.D.	Medical Director, USPHS	LBP NIDDK
Other:	Sushil K. Taneja, Ph.D.	Visiting Associate	LBP NIDDK

COOPERATING UNITS *(if any)*

Dr. H. Tabor, LBP, NIDDK

LAB/BRANCH

Laboratory of Biochemical Pharmacology

SECTION

Section on Pharmacology

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.4	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have continued our studies of polyamines in Saccharomyces cerevisiae, utilizing biochemical and genetic methods.

We have emphasized the study of the gene for S-adenosylmethionine decarboxylase, an essential enzyme in polyamine biosynthesis, in Saccharomyces cerevisiae. This protein is also important since it belongs to a small group of enzymes that have a unique requirement for activity, namely, a covalently-linked pyruvate. We have prepared a 3200-bp fragment of yeast DNA that can be maintained stably in a shuttle yeast vector. This fragment of DNA contains the complete coding sequence for the enzyme and is expressed in yeast. We have evidence for the synthesis of a longer proenzyme that is cleaved to form an active pyruvate-containing subunit. We are sequencing the gene using the dideoxy technique in order to study the mechanism of formation of the essential pyruvate that is covalently linked to the protein.

We have shown that a cell division cycle defect occurs in amine-deficient cells, with the accumulation of budded forms. The more complete the amine deficiency, the more nearly equal the size of the mother cell and the bud. Thus we have shown that polyamine deficiency is associated with arrest of the cycle in a late period, just before cytokinesis.

Ornithine decarboxylase, the enzyme that synthesizes putrescine in S. cerevisiae, has now been purified (75% pure) as an 86,000 molecular weight polypeptide under conditions that minimize proteolysis. We find that the enzyme is synthesized as an 86,000 molecular weight polypeptide that is easily and specifically converted to the 68,000 molecular weight form by proteolysis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 24,710-36 LBP
(formerly Z01 AM 24,710-35 LBP)

PERIOD COVERED
October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Polyamine Biosynthesis and Function in Escherichia coli

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Herbert Tabor, M.D. Supervisory Medical Officer (Research); Chief, Section on Pharmacology, LBP; and Chief, Laboratory of Biochemical Pharmacology LBP NIDDK

Other: Qiao-Wen Xie, Ph.D. Visiting Fellow LBP NIDDK

COOPERATING UNITS *(if any)*

Dr. C. W. Tabor, LBP, NIDDK

LAB/BRANCH
Laboratory of Biochemical Pharmacology

SECTION
Section on Pharmacology

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.6	2.6	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have continued our studies of the genetics and regulation of the biosynthesis of spermidine in E. coli, emphasizing the two enzymes involved, S-adenosylmethionine decarboxylase and spermidine synthase. We are particularly interested in S-adenosylmethionine decarboxylase, both because it is essential for spermidine synthesis and because it requires covalently-linked pyruvate for activity. We are also very interested in spermidine synthase, since no previous information was available on the genetics of this enzyme.

In the current studies we have shown that the gene for spermidine synthase (speE) lies immediately adjacent to and upstream to the gene for S-adenosylmethionine decarboxylase (speD). Both genes are controlled by a single promoter upstream to the speE gene. Thus, these two genes form an operon and the genetic regulation of the speD gene is intimately dependent on the speE gene and its promoter.

We have purified both proteins to homogeneity, and Dr. Darrell T. Liu (DBB, CDB) has carried out partial protein sequencing. We have also sequenced the DNA coding for these enzymes and now have shown, by the identity of the amino acid sequences deduced from the DNA sequences with those of the protein fragments, that each gene codes for the respective structural protein.

We have shown that S-adenosylmethionine decarboxylase is formed as a proenzyme, which is then cleaved to produce two smaller fragments. We have identified the specific peptide bond that is cleaved to form the pyruvoyl group as a lysylserine linkage.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 DK 24,940-13 LBP

(formerly ZO1 AM 24,940-12 LBP)

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Killer Double-Stranded RNA Plasmids of Saccharomyces cerevisiae

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Reed B. Wickner, M.D. and Chief, Section on Genetics of Simple Eukaryotes, LBP	Medical Director, USPHS,	LBP NIDDK
Others:	Tsutoma Fujimura, Ph.D.	Visiting Associate	LBP NIDDK
	Tateo Icho, Ph.D.	Visiting Associate	LBP NIDDK
	Hyun-Sok Lee, Ph.D.	Visiting Fellow	LBP NIDDK
	Yang-Ja Lee, Ph.D.	Guest Researcher	LBP NIDDK
	M. Rosa Canibano Esteban, Ph.D.	Guest Researcher	LBP NIDDK

COOPERATING UNITS *(if any)*

Faculte des Sciences Agronomiques de l'Etat, B5800, Gembloux, Belgium

LAB/BRANCH

Laboratory of Biochemical Pharmacology

SECTION

Section on Genetics of Simple Eukaryotes

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.2	4.8	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Yeast has 5 families of double-stranded RNAs (dsRNAs) replicating in its cytoplasm. These are called L-A, L-BC, M, T, and W. L-A, L-BC, and M are found in intracellular, noninfectious virus-like particles (VLPs). M encodes a secreted toxin and immunity to that toxin, while L-A encodes the major protein of the VLPs in which both itself and M are encapsidated. We have established an *in vitro* replication system for L-A, L-BC, and M. We find that (-) strand L-A synthesis occurs in VLPs carrying only the (+) strand, while (+) strand synthesis occurs by a conservative mechanism in particles carrying L-A dsRNA. New (+) strands are extruded from the VLPs. M replication occurs *in vitro* by a mechanism similar to that of L-A except that, because M is less than half the size of L-A, the same particles that can only hold one L-A can hold one or two M molecules. [D], a new cytoplasmic genetic element we discovered, exacerbates the disease produced by the derepression of M dsRNA replication seen in *ski* mutants. We have isolated chromosomal mutants unable to maintain [D] (*mad*). The *MAK* genes are chromosomal genes essential for M dsRNA replication, while the *SKI* genes are repressors of this process. We have isolated clones of *MKT1*, *MAK11*, *MAK16*, *MAK18*, *SKI3*, *SKI8*, and *CDC16*. We have completely sequenced the *MAK11*, *MAK16*, and *CDC16* genes.

ANNUAL REPORT OF THE

LABORATORY OF CHEMICAL BIOLOGY
NATIONAL INSTITUTE OF DIABETES, AND DIGESTIVE AND KIDNEY DISEASES

The Laboratory of Chemical Biology conducts research on fundamental problems in molecular interactions, relating forces and molecular assembly to cell function; on structure, function, and dynamics of proteins; and on molecular biology and genetics, especially as related to genetic disease. The Laboratory has recently initiated several major new areas of research. There is currently a large program to identify and isolate the trans-acting factors in the nuclei of human erythroid cells that control the ontogeny of hemoglobin synthesis. A related research endeavor focuses on ascertaining the way in which genetic variables, especially fetal hemoglobin levels and co-existing alpha-thalassemia, affects the manifestations of sickle cell disease. Also at the molecular genetic level, are studies to identify new genes coding components of the human T-cell receptor and a search for the existence of possible pathogenic retroviruses in human lymphoid diseases. One component of the work on protein folding is now concentrating on the production of monoclonal antibodies to yeast cytochrome c so as to be able to study the forces stabilizing antigen-antibody interactions. At the biophysical level are analyses of hydration forces among macromolecules, of the effects of mechanical motion on the interactions of membranes, and of the flexibility and conformation of oligonucleotides and DNA-gyrase complexes. At the cell biological level of analysis, studies are underway on the mechanisms of membrane fusion and exocytosis as related to osmotic swelling and of the opening and closing of membrane channels in response to osmotic stress forces. Towards the end of this reporting year, a long term project was initiated to develop a true animal model of sickle cell anemia, using transgenic techniques.

During the last year the reorganization of the Laboratory that had been initiated five years ago, but developed more fully in the last three years, has been largely completed. There are now three newly renamed sections: the Section on Molecular Forces and Assembly, the Section on Protein Chemistry and Conformation, and the Section on Molecular Biology and Genetics. The Section on Molecular Forces and Assembly, under Dr. V. Adrian Parsegian (also of the Physical Science Laboratory of the Division of Computer Research and Technology) is concerned with biophysical and cell biology studies of the forces between DNA, protein and lipid molecules, especially as these forces affect biological membrane structure and properties. The Section on Protein Chemistry and Conformation, under Dr. Hiroshi Taniuchi, is devoted primarily to the study of protein folding and dynamics, in particular to the origin of forces stabilizing the three dimensional structure of globular proteins. The Section on Molecular Biology and Genetics, under Dr. Alan N. Schechter, is concerned primarily with the molecular genetic basis of the developmental control of gene expression, especially in human erythroid and lymphoid cells, and its relevance to the understanding of the molecular basis of disease states and possible approaches to their therapy.

During last year, Dr. Constance Tom Noguchi has received tenure as a Research Physicist and independent investigator in the Labortory of Chemical Biology. Dr. David I. Cohen has joined the Laboratory as a Senior Medical Staff Fellow to establish a program in the molecular genetics of normal and abnormal human lymphoid cells. Dr. Griffin Rodgers is now serving as a Robert Wood Johnson Fellow. Dr. Edward Steers has formally left this Laboratory to become Deputy Scientific Director of the Intramural Research Program of NIDDK. Dr. Anfinsen, who is a Scientist Emeritus in this Laboratory, visits here several days each month, in part in his additional capacity as senior advisor to the medical students in the Howard Hughes Institute Program at the NIH.

Extensive research collaborations exist within this Laboratory and with other Laboratories in this Institute, in NIH, and nationally and internationally as outlined in the individual Research Project Reports. Formal collaborations include the sharing of personnel and financial resources between NIDDK and DCRT in the establishment of the Section on Molecular Forces and Assembly, sharing of personnel for Dr. David Cohen's program with Dr. Alfred Steinberg of the Arthritis and Rheumatism Branch of NIAMS, and sharing of personnel for the erythroid biology research with the Molecular Hematology Branch of NHLBI. In addition, a formal collaboration has been established involving the exchange of personnel and resources with the Laboratory of Experimental Hematology of the Armed Forces Radiobiological Research Institute at the National Naval Medical Center. The participation of this Laboratory in the NIH Inter-Institute Medical Genetics Program and the NIH-George Washington University Hematology Training Program continues to grow.

Section on Molecular Forces and Assembly

The work of this Section has involved both theoretical and experimental analyses of forces stabilizing DNA, proteins, lipids, and membranes. Theoretical analyses have clarified the mechanical properties of non-lamellar lipid structures involved in membrane fusion events and the effects of mechanical motion on the long range forces acting between neighboring membranes or linear macromolecules. Hydration forces between DNA molecules have been measured using the osmotic stress technique with X-ray diffraction measurements of molecular spacing. These results show that the changes in entropy in the energetics of these interactions are determined by the structure of water of hydration surrounding these molecules. Other experimental studies show that the bending of oligonucleotides is due to perturbations of the hydration of the groove in the DNA and that the source of entropy that drives the conformational change from B to Z forms of DNA is related to the release of two water molecules per base pair. It has also been shown, using electric dichroism measurments, that ATP binding to DNA-gyrase complexes causes the DNA tails to fold back across the complex. These studies are of major importance in emphasizing the role of water and hydration forces in macromolecular interactions.

Measurements of conductance in membranes, both natural and model systems, as a function of osmotic stress, indicate that the opening and closing of such channels occurs not as a gating phenomenon but as a major closure of the entire channel space. Reconstitution and purification studies have allowed the improved isolation of such channels in vesicles for further characterization. The mechanism of exocytosis in sea urchin eggs has been studied by a variant of the osmotic stress method. The experiments are allowing clarification of the contribution of osmotic swelling, specific ion fluxes, and other components to the mechanism of exocytosis. More direct results have come from studies of the

large secretory granules in the beige mouse mast cells using an array of simultaneous physiology and structural measurements. These results show that fusion of the secretory granules preceeds swelling of the vesicles. This suggests that vesicle swelling is not needed for membrane fusion, but may be required for release of products. In related studies, evidence for the role of a G protein in the release of calcium from rat lacrymal glands in response to acetylcholine was obtained.

Section on Protein Chemistry and Conformation

The recently completed studies on folding and fragment complexes of staphylococcal nuclease RNase A, cytochrome c and certain chemically synthesized derivatives of cytochrome c has led to the hypothesis of the importance of globally coupling forces involved in the folding of proteins. It is suggested that these forces do not correspond to the conventional ones studied in protein physical chemistry but constrain individual atomic residues in the three dimensional structures of proteins and are detected by changes in the same direction of both enthalpy and entropy upon substitutions of specific amino acids. These coupled forces are being studied in analyses of derivatives of cytochrome c with respect to ligand binding and other properties. They could constitute a significant new way of examining the structure and dynamics of proteins.

An outgrowth of the above work is an extensive project on the total chemical synthesis of cytochrome c and its derivatives, including a postulated ancestral sequence. A variety of synthetic strategies have been developed for coupling large and small fragments of the protein made by Merrifield solid phase methods and for the eventual covalent linking of the heme group with a specific enzyme that has already been characteried. Another related project is the production and characterization of a number of monoclonal antibodies to cytochrome c. The monoclonal IgG molecules are being characterized as to their sites of binding to the protein in preparation for studies of the dynamics of their interaction to native protein as well as to chemical derivatives and fragment systems.

Section on Molecular Biology and Genetics

The major part of this Section's work is devoted to clarifying the molecular genetic basis by which the developmental switch from embryonic to fetal to adult hemoglobins occurs in the human. Understanding of the control of globin gene expression would be a very important general point with respect to developmental biology, but might also have specific therapeutic relevance for the diseases of hemoglobin. The project is being pursued for the most part by trying to understand the phenotype of a cell line, the K562 cells, which appears to be arrested in the late embryonic stage of globin gene expression. Evidence has been obtained that there are intranuclear factors, *trans*-acting factors, that determine which genes are expressed and which are silent in these cells. During the last year, a broad range program to identify and isolate these factors and to understand their mechanism of action has been developed. To this end studies are underway of nuclease hypersensitivity in the chromatin structure around active and inactive globin genes, of the structure and function of the globin promoter regions (*cis*-acting) by fusing these to the gene for the enzyme chloramphenicol transferase (CAT) and assaying CAT activity in cells transfected with various promoter-CAT fusion genes, of *in vitro* transcription systems to provide a direct assay for *trans*-acting factors, and of the effects of known viral *trans*-acting factors (such as the SV40 T antigen, the adenovirus E1a

protein, the HTLV I tat-1 gene and the products of various oncogenes) to clarify the mechanism and specificity of trans-activation. In addition direct binding assays and subtractive cloning techniques are being used in order to isolate the protein or the gene for one or more of these trans-acting factors. Although these goals are not simple, the elucidation of the control of this biologically and medically important human gene system would be a potentially major step in molecular and developmental biology and in applied medical molecular genetics.

This Section also continues its work on the pathophysiology of sickle cell anemia. During the last year the role of red cell heterogeneity, alpha thalassemia and fetal hemoglobin levels to determining disease severity and expected response to therapy has been clarified. Studies of non-invasive methods to evaluate blood flow in sickle cell anemia patients also continue to offer the potential of developing objective measures of disease severity. A project to develop a true animal model of sickle cell disease by using transgenic methods to introduce the β^S and the human α gene into mice has recently been initiated. Methods to remove the endogenous mouse globins, including the use of α- and/or β-thalassemic mice or the use of anti-sense globin genes, are also being studied. This work is regarded as a long term project to develop a true model of the disease for study of sickle cell rheology, pathophysiology, and treatment.

A new program in the Section is the study of genes in human lymphoid tissues. Genes that may code for parts of the T-cell receptor, other than the α and β chains, are being studied. These genes are postulated to play a major role in the mechanism by which an organism distinguishes between "self" and "non-self." An experimental model in which autoreactive T cells occur after haploidentical bone marrow transplantation is being analyzed with respect to rearrangements and expression of the T-cell receptor genes. Understanding the mechanism of self recognition should ultimately lead to improved allograft survival, at a time when organ transplantation is assuming a continually increasing role in the therapy of hematopoietic, liver, kidney, and cardiac disease. Lastly, studies are underway using viral molecular probes in a variety of disease states that involve lymphoid tissues on the hypothesis that some of these may be caused by previously unknown retroviruses. Although the viruses may not be detectable intact in these cells, DNA fragments may persist in the genome. These studies illustrate several applications of current molecular genetic techniques to important medical problems.

SECTION ON MOLECULAR FORCES AND ASSEMBLY

I. Molecular Forces

A. Molecular Forces

The PI, Sol Gruner and Peter Rand have succeeded in determining the mechanical properties of a non-lamellar lipid phase, the inverted hexagonal form thought to correspond to transient structures during membrane fusion. It appears that different combinations of lipids have different most-favored or "intrinsic" radii of curvature. The work of forced deviations from this configuration can be described in terms of a bending modulus whose value is nearly the same for all lipids. It also seems that in these non-lamellar forms the hydrocarbon chains are under little strain but rather act to fill space. Hydrocarbons added to bilayers will tend to drive the lipids to non-lamellar forms.

These lines of physical reasoning lead immediately to better understanding of the reasons for the biochemical metabolic steps in lipid metabolism. It also provides a strong indication for a morphological role for hydrocarbons such as dolichol known for its biochemical function anchoring oligosaccharides during synthesis.

With Evan Evans and Donald Rau, the PI has further pursued the problem of relating the mechanical motion of neighboring membranes or linear macromolecules to the long-range forces acting between them. We have used a measure of extent of molecular motion from the widths of x-ray diffraction peaks scattering from arrays of condensed DNA together with direct force measurements to see how forces and motion affect each other. (Parsegian)

B. Hydration Forces and Applications of the Osmotic Stress Technique

Hydration forces are the recently uncovered interactions between DNA or lipid bilayer surfaces that dominate the energies between these surfaces at separation distances of 20Å and less. These forces appear to be due to the structuring of water between surfaces and can be either strongly attractive or strongly repulsive depending on the surface hydration. We can directly measure these forces by combining the osmotic stress technique with x-ray diffraction to measure the separation of the surfaces. In analyzing the energetics of these forces, we have concentrated on the osmotic pressure induced assembly of Mn^{2+}-DNA. The force curves show an abrupt transition at a critical osmotic pressure, that depends on temperature and Mn^{2+} concentration, between repulsive and attractive hydration forces, mediated by the presumed rearrangement on Mn^{2+} on the surface of DNA. The transition is entropically driven presumably by the release of bound water. We have now examined the effect on the transition of different anions that structure bulk water differently. The transition occurs more readily with ClO_4^- than with Cl^- and is more difficult with SO_4^{2-} than with Cl^-. We have quantiated the entropy changes and found that the differences are due to the additional entropy gained or lost by releasing structured water around DNA helices into the bulk. This is the first clear, direct indication that water structuring is the source of hydration forces. (Rau, Parsegian)

C. Structure and Physical Properties of DNA and DNA-Protein Complexes

Progress has been made in physically characterizing three distinct systems, all of which have possible biological significance. A substantial difference in the binding energy or flexibility has been found between poly (dG-dC) and its methylated analogue, poly (dG-M^5dC). This difference makes it probable that resistance to bending is largely due to perturbations in groove hydration rather than in base stacking interactions. It also suggests that a biological effect of CpG methylation is to potentiate the ease of nucleosome formation relative to protein factor binding.

We have identified the source of entropy, that drives the conformational transition from the B to Z forms of poly(dG-m^5dC), as the release of two water molecules per base pair. This result suggests that this transition will be signficantly easier in the cell, under conditions of osmotic stress, than in dilute solution.

The conformational change that occurs with ATP binding to gyrase-DNA complexes has been determined. The DNA tails, that extend out from the core in the basic complex, fold back cross the protein when ATP is added. These results will enable us to correlate mechanism, biochemistry, and structure for the supercoiling reaction of gyrase. (Rau)

II. Membrane Transport and Exocytosis

A. Control of Membrane Transport by Osmotic Stress

To measure the internal volume change during opening and closing of ionic transmembrane channels, we have been subjecting perfused preparations to positive and negative osmotic stress. The extra work of channel opening under osmotic stress is measured as a shift in the current-voltage curve or as a bias in the open/closed statistics of a channel. Suppression and enhancement of potassium channel conductance in the squid giant axon correspond nicely to the response one expects to osmotic stress. The data do not fit a simple blocking model. The channel volume infered has an upper bound of 1300 cubic angstroms. The mitochondrial voltage-dependent anion channel (VDAC) inserted into planar lipid bilayers shows a volume change of 20 to 40 thousand cubic angstroms. These are large changes inconsistent with traditional blocking or local gating models but supporting models with major closure of the channel space. A microcomputer data analysis system has been further adapted for these measurements.

The gap junction is the locus of direct transfer of ions and small molecules from cell to cell. We have (1) incorporated material from isolated gap junctions into vesicles, (2) applied a density shift technique to select vesicles containing large open channels, and (3) incorporated those channels into planar bilayers. A transport-specific purification of vesicles containing channels has been developed to improve the efficiency of the reconstitution. Predominantely three sizes of conductance changes were seen. They are 20-30, 60-70, and 110-130 pS, each having different degrees of voltage sensitivity, asymmetry, and kinetics. Membranes containing several channels were anion selective. (Zimmerberg, Parsegian)

B. Control of Exocytosis in Sea Urchin Eggs by Osmotic Stress

We have continued to examine the role of osmotic swelling of secretory granules during exocytosis. This year, we are focusing on exactly how this swelling is accomplished. We examine sea urchin egg cortical granule exocytosis with differential interference contrast light microscopy, phase contrast microscopy, fluorescence microscopy, and intracellular recording to measure cell capacitance and potential. Compromising the integrity of the secretory granule membrane to the extent of allowing free passage of small molecules does not alter calcium stimulated secretion in vitro. Exocytosis proceeds without ions. Granules remain intact in the presence of concentrations of digitonin sufficient to cause lucifer yellow entry. Higher concentrations of digitonin cause the same phase-transition of contents seen with calcium. This phase transition can be inhibited reversibly with osmotic stress. Exocytosis is prevented by including a variety of polymers of different chemical composition and molecular weight in the sea water surrounding the eggs. The increase in membrane capacitance which occurs during exocytosis is not greatly altered by inhibitory concentrations of polymer. Calcium and magnesium reduce the osmolality required to prevent exocytosis. These results suggest that calcium causes swelling by an

alteration in the state of the internal granule phase to increase the affinity of this phase for water rather than by inducing ionic fluxes into the granule. (Zimmerberg, Parsegian)

C. Histamine Release from Beige Mouse Mast Cells

We are continuing to study the mast cell of the beige mouse, an animal model for the Chediak-Higashi syndrome in man. We study this cell because of its very large secretory granules, like the large granules of the neutrophils in the diseased state. One defect in that immuno-suppressed syndrome is failure in secretion. The large size of the beige mouse mast cell make possible detailed analysis of their exocytosis to the plasma membrane. Last year, we developed an instrumental array capable of simultaneous physiologic and anatomic real-time measurements of living cells with control of the internal millieu, using it to measure the capacitance of secretory and phagocytic peritoneal cells from the internally perfused mast cell from the beige mouse. Thus, we can combine biochemistry, anatomy, and physiology by performing perfusion studies on single active cells while recording both optical information (image, exocytosis, endocytosis, contraction), and electrical information (ionic currents, voltage clamp, capacitance) with 17 msec resolution. Preliminary studies reveal that the fusion of the secretory granule preceeds the swelling of that vesicle by 17-150 milliseconds. Granules which have been shrunken by hyperosmotic solutions also fuse to the cell membrane well before detectable swelling. Apparently in this system vesicle swelling is not needed for membrane fusion but may be required for the release of secretory products. (Zimmerberg, Curran)

D. G-protein Diffusion During Muscarinic Activation

We have studied the muscarinic response of rat lacrymal gland cells to acetylcholine with the tight-seal whole-cell technique. To measure the liberation of calcium from internal stores in that cell we monitored the calcium dependent K and C1 currents resulting from application of acetylcholine. Intracellular inclusion of IP2 liberated calcium. To show that polyphosphoinositide (PIP2) hydrolysis is a step in muscarinic activation we added neomycin, which binds to PIP2 and blocked activation by acetylcholine. The response to agonist diminished over several minutes after initiation of whole-cell dialysis. We found that the reponse was stable for some minutes, then decreased exponentially. The delay and time constant of the washout was directly proportional to the cell volume. It appeared that some soluble intracellular factor, needed after receptor activation, was needed for liberation of intracellular calcium. Attempts to constantly replenish PIP2 by inclusion of CTP, ATP, and inositol did not halt washout. Intracellular inclusion of ATP, GTP, cAMP, cGMP, together or alone, did not stop washout. We observed washout in one cell, gently removed the pipette after no further response to acetylcholine was seen, and sealed to the same cell a new pipette containing IP3. A rapid and sustained response was seen upon initiation of the whole cell mode. Intracellular inclusion of GTP-S potentiated the muscarinic response and slowed washout. We conclude that the washout is due to the loss of a diffusible factor which acts after muscarinic receptor activation and before polyphosphoinositol release. We suspect the action of the factor to involve a G protein. (Zimmerberg)

SECTION ON PROTEIN CHEMISTRY AND CONFORMATION

I. Protein Folding and Dynamics

A. The Mechanism of Protein Folding: Global Coupling - A New Type of Interaction.

The studies of the fragment complexes of staphylococcal nuclease, RNase A and cytochrome c have led us to the hypothesis that after folding of almost the entire polypeptide chain (including the S-S bonds in the case of RNase A) the interatomic interactions would be globally coupled to generate extra force for shifting the equilibrium of folding and unfolding in favor of folding. To understand this extra force and speculating that some of evolutionarily invariant amino acids might play a role we have investigated the effect of substitution of invariant proline 30, leucine 32 and glycine 34 and partially invariant Leu 35 of cytochrome c using the three-fragmnt complex of horse cytochrome c as described in the previous years. The first phase of this work is now complete with important results: (1) The extra force would not be van der Waals interaction, hydrogen bond, hydrophobic interaction or electrostatic interaction per se, i.e. this force would be a new type of interaction having a property of delocalization; (2) The extra force would constrain the atomic positions of individual residues in a concerted manner throughout the structure; (3) The extra force would be detectable on the basis of perturbation of enthalpy and entropy changes (the same sign) associated with folding by substitution of some specific amino acid such as evolutionarily invariant one after taking into account the contributions of possible perturbation of the unfolded form. In the case of cytochrome c the extra force would be stronger for the reduced form than for the oxidized form thus modulating the redox potential. Further, the studies of hybrid complexes using fragments from horse, tuna, Candida krusei, and yeast cytochromes c have indicated that the information for the cytochrome c fold is exchangeable between the fragments of phylogenetically distant species as measured by ligation of Met 80 to the heme iron, and suggested that; (a) however, mutation of a few specific amino acids or perhaps a single amino acid could alter stabilization of the Met 80-S-heme-Fe bond; (b) such destabilization could be reversed by mutation at some other position or positions. (Taniuchi, Lisowski, Fisher, Truong)

B. Chemical Synthesis of Cytochrome c: The Roles of Individual Residues

The mitochondrial cytochromes c of the present species are presumed to collectively reflect evolutionary events occurring for the last 1.5 billion years. These evolutionary secrets, if uncovered through finding the roles of the invariant residues, may help understanding the principles underlying structure-function of proteins. In this context, it would be worthwhile to chemically synthesize the ancestral form of cytochrome c predicted by W.M. Fitch and E. Margoliash and test whether it is active. The feasibility of this project is supported by our finding that the complementing fragments are completely exchangeable between horse and Candida krusei cytochromes c for formation of hybrid complexes (see the other report). As a first step, we plan to synthesize horse cytochrome c via synthesis of apocytochrome c, using the Merrifield solid phase method and joining two apofragments (complexation assisted rejoining, see the previous report), followed enzymatic attachment of heme, using cytochrome c synthase found in this Section. In extending the joining procedure we have shown that apofragment [Hse>-65](23-65) (homoserine-lactone), but not [Hse>-65](39-65), of horse cytochrome c rejoins to apofragment (66-104) in the pres-

ence of reduced heme fragment (1-25)H and that intact methionine 80 is essential for this rejoining reaction. On the basis of these results, to make semisynthetic [Hse-65](28-104), we carried out synthesis of fragment [Gly-66](28-66) of horse cytochrome c, containing tryptophan at position 59.

In the effort to develop a second procedure for joining of fragments, we found a novel carboxypeptidase A catalyzed transpeptidation as follows. Synthesized radiolabeled tripeptide Leu-Met-His-amide or Leu-Arg-Met-amide and excess of heme fragment [Hse-69](1-69)H of yeast cytochrome c or [Hse-65](1-65)H of horse cytochrome c were incubated with carboxypeptidase A in the presence of 50 to 70% glycerol at pH 10.0 at 20 to 30°C. Analyses of the product indicated that both labeled tripeptides replaced the carboxyterminal homoserine with the efficiency up to 50% after 7 days. (Taniuchi, Gozzini, Du, Fisher)

C. Origin of the Specificity of Antigen-Antibody Interaction

The studies in this Section have led us to the hypothesis that the energy state of one region of the structure of proteins would be coupled with those of distant regions to generate extra stabilizing energy. Thus, to investigate whether the strength of antigen binding is related to the reduction of the fluctuation of distant regions of the antibody by such coupling, we have developed 5 hybridoma cell lines, 4.74.6, 4.128.6, 4.145.10, 2.96.12 and 2.34.19 producing monoclonal IgG1 directed against yeast iso-1-cytochrome c. Monoclonal IgG1 4.74.6, 4.128.6 or 4.145.10 reacts with yeast cytochrome c but not with cytochrome c of Candida krusei, tuna, chicken, pigeon, rat, sheep, dog porcine, bovine or horse. Monoclonal IgG1 2.96.12 or 2.34.19 reacts with both yeast and C. krusei cytochromes c. The former IgG1, but not the latter also reacts with tuna, rabbit and chicken cytochromes c in a lesser extent. None of these five monoclonal antibodies reacts with the apocytochromes c tested (yeast, C. krusei and horse). Monoclonal IgG1 4.74.6, 4.128.6 or 4.145.10 does not react with heme- or apofragments of yeast cytochrome c but does react with the productive fragment complexes of yeast cytochrome c or even with the hybrid complexes containing the horse heme fragment and yeast apocytochrome c. On the basis of tests with hybrid complexes, the antigenic determinants for these three antibodies appear to be located in residues 59 to 108. Monoclonal IgG1 2.96.12 and 2.34.19 do not react with any of these complexes or hybrid complexes. The results indicate that all of these five monoclonal antibodies recognize the three-dimensional configuration of the antigenic determinant formed by folding of the native cytochrome c (and also the complexes in the three former cases). In addition, the three former antibodies more specifically recognize the side chains of the amino acids in the determinant than the two latter. However, the two latter antibodies discriminate between the native protein and the fragment complex but the three former do not. (Taniuchi, Silvestri, Fisher)

SECTION ON MOLECULAR BIOLOGY AND GENETICS

I. Developmental Control of Hemoglobin Synthesis

A. Chromatin Structure and Trans-activation of Human Globin Genes

Chromatin structure and trans-activation are two important aspects of gene regulation. Chromatin from actively transcribed genes exhibits a structure different from that of non-transcribed genes. It has been shown that trans-acting proteins can bind to DNA and confer a unique chromatin structure, and such a protein DNA interaction has been suggested to play an important role in

gene regulation. In an attempt to investigate the mechanisms of regulation of human globin gene expression two approaches have been used: 1) We have studied the chromatin structure around the human beta-globin gene in K562 cells by using DNase I and S1 nuclease. Despite the beta-globin gene not being expressed, we found nuclease hypersensitive sites in both its 5' and 3' flanking regions. While the 3' hypersensitive sites are identical to that of active beta globin genes, the 5' hypersensitive sites shift further upstream. This suggests that the lack of beta-globin expression in K562 cells may be related to unique aspect(s) of the chromatin structure in the immediate 5' flanking region of the beta-globin gene. 2) To investigate the mechanism of trans-activation, globin promoters were tested in a transient expression system. We found that the globin promoter can be activated by SV40 T antigen in monkey kidney CV-1 cells. This trans-activation can be further stimulated by including a SV40 enhancer in the globin plasmid. The mechanism of the trans-activation is currently under study. (Cao, Elion, Mishoe, Schechter)

B. Factor(s) Controlling Globin Gene Expression in K562 Cells

K562 cells are used as a model to study the control of human globin gene expression. They express embryonic and fetal but not adult globin genes. Results suggests that the lack of transcription of the adult beta-globin gene results from an alteration of a regulating trans-acting factors(s). Our objectives are to provide evidence for the existence of such a factor, to elucidate its nature as an activator or a repressor and to set up an in vitro assay for its measurement. Globin promoters activity is deduced from the transient expression of the bacterial CAT enzyme in K562 cells transfected with plasmids in which transcription of the CAT gene is driven by various globin gene promoters. In competition experiments, cells have been cotransfected with these plasmids and an excess of the corresponding globin promoter. However, low efficiency and poor reproducibility of transfection impaired analysis of the results. Therefore, details of the transfection procedure have been thoroughly worked out to optimize reproducibility and efficiency. Since competition experiments required final DNA concentrations found to be inhibitory of CAT expression in control experiments, we are now constructing competitor plasmids containing multiple copies of the globin promoter sequences. Experiments conducted with K562 cells grown in the presence of 5-azacytidine, did not provide evidence for expression of the CAT plasmid containing the beta-promoter, but showed non-specific enhancement of expression of the other CAT plasmids. Finally, we are attempting to develop an assay for trans-acting factors that would allow us to track them in a purification scheme. We constructed SP6 promoter-containing plasmids in which the globin promoters are used as templates for in vitro transcription. We hope to be able to set up conditions in which binding of proteins to the promoter will result in premature transcription termination. Labelled transcripts could then be analyzed by polyacrylamide gel electrophoresis and autoradiography. (Elion, Berg, Cao, Schechter)

C. Regulation of Globin Gene Expression by 5' DNA Sequences

Previous studies from this laboratory have shown the molecular defect in K562 cells appears to be in a trans-acting factor. The most likely possibilities are that a repressor is continuously synthesized which binds to DNA near the beta-globin promoter, preventing transcription, or that an activator which is necessary for beta-globin transcription is not synthesized, or that K562 cells both synthesize a repressor and lack an activator. To test these hypotheses we are using a plasmid, p-beta-GLCAT, which contains the human beta-globin

promoter and additional 5' DNA fused to an easily assayable gene, CAT (chloramphenicol acetyl transferase). The fusion gene, beta-CAT, is inactive in K562 cells so we are attempting to activate it using several approaches. Our experiments to activate beta-CAT either in cis, with the SV40 enhancer, or in trans, with the E1A protein, have been unsuccessful in K562 cells but successful in Chinese hamster cells, consistent with the presence of a repressor in K562 cells. Further support for this model comes from 5' deletion mutants of beta-CAT, which indicate that deletion of certain 5' sequences does allow beta-CAT expression in K562 cells. Thus, there seems to be a negative regulatory region near the beta-globin promoter. Experiments are underway to try to identify the DNA binding site of the putative repressor and to identify the repressor molecule itself. (Berg-Lovett, Gong, Elion, Qian, Schechter)

D. Isolation of Embryonic Globin Transcriptional Factors by Subtractive cDNA Cloning

The transcription of human globin genes may involve the complex interaction of a variety of factors. The K562 human erythroleukemia cell line can serve as a model for the study of globin gene expression. The K562 cell line can be induced by hemin to accumulate embryonic and fetal hemoglobin, but not adult hemoglobin. It has been demonstrated that the beta-globin gene is intact but inactive in these cells. The cloned K562 beta-globin gene is expressed in COS cells and the transcriptionally inactive beta-globin gene in HEL cells is activated in MEL x HEL hybrids suggesting that the induction of beta-globin gene expression perhaps requires a specific transcriptional factor. The zeta-globin gene promoter functions after microinjection into oocytes but not after transcription into HeLa or COS cells also suggesting that there might be transcriptional factors specific for embryonic genes.

The current study assumes that induced K562 cells contain transcriptional factors specific for embryonic globin genes, which are absent or present only at low levels in uninduced K562 cells. We are preparing cDNA from the mRNA of induced K562 cells. The mRNA from uninduced K562 cells will be used to subtract the background corresponding to proteins present in both the induced and uninduced K562 cells. The remaining cDNA will be cloned into a vector (lambda gt10) to replicate enough copies for screening with mRNA or cDNA, from both the induced and uninduced K562 cells. Those cDNA clones which are differentially expressed will be further characterized by transfecting back into K562 cells or other hemoglobin or non-hemoglobin producing cell lines, or by inserting into a protein expression vector (lambda gt11) so that the protein can be examined. (Wu, Wada, Torain, Noguchi)

E. Transcription of Globin Genes In Vitro

We are working to establish an in vitro transcription system in which the transcription pattern of the globin genes mimick that observed in vivo, i.e. expression of embryonic and fetal, but no adult globins. As an initial approach, in vitro transcriptional analysis using a whole cell and nuclear extract made from K562 cells was carried out. We have demonstrated that all cloned globin genes (beta, epsilon, and gamma) are transcribed in vitro indicating a lack of differential transcription of these genes. Entertaining the possibility that differential transcription of genes may require some type of DNA conformation, K562 chromatin was used as a DNA template. While the results are still preliminary, we find that proper transcriptional control similar to that observed in vivo can now be demonstrated. These results were obtained by

S1 nuclease mapping wherein we observed in vitro transcription of the fetal but not adult globin genes. We are currently exploring several approaches to increase the sensitivity of our transcription assay to insure that this important observation is not a result of our inability to detect adult globin transription. These inluce utilization of a K562 nuclear extract for transcription, altering transcription conditions so chromatin is more accessible to transcription factors, and using high specific activity SP6 globin RNA probes to detect accurate transcription. The establishment of an in vitro transcription system which mimicks the K562 transcription pattern observed in vivo will enable us to examine molecules which surround a gene and its control region when that gene is in an active state and when it and other genes are in a repressed state. (Mishoe, Schechter)

F. Transcriptional Control of Globin Genes in In Vitro Assays From K562 Cell Extracts

It has been demonstrated that K562 cells express epsilon- and gamma-globin genes but do not express beta-globin genes. The regulation of gene expression occurs at the level of transcription. We have set up a cell-free in vitro transcription system from K562 cells to determine the requirements for globin mRNA synthesis. As an initial attempt, we have prepared extracts from nuclei of both hemin-induced and uninduced K562 cells. The nuclear extracts could direct accurate transcription initiation in vitro from the epsilon-globin gene promoter without supplement with whole cell extracts. The results up to the present point to the existence of a globin gene expression regulatory factor(s) in K562 nuclear extracts. The in vitro transcription system will be used as an assay system for the isolation and characterization of such factors. (Wada, Mishoe, Torain, Noguchi)

G. Effects of HTLV-1 Tat Gene Product on Globin Expression

The control of human globin gene expression in erythroid cells involves trans-factors (substances active at multiple locations in the genome), which have yet to be identified or clearly described. On experimental approach to their identification is to study the effects on globin gene expression of well-described trans-factors from tumor viruses. Trans-factors from adenovirus 5 and pseudorabies virus are known to stimulate beta-globin transcription. HTLV-I specifies a similar trans-factor, tat 1, whose effect on non-viral genes is not known. We have designed three lines of transfection experiments to test the interaction of tat 1 and globin genes: (1) a normally inactive beta-globin plasmid is transfected into C81-66-45 cells, which produce tat 1. Detection of beta-globin RNA would suggest trans-activation of this gene by tat 1. (2) nonerythroid cells are cotransfected by a tat 1 expression vector and a beta-globin plasmid. Expression of beta-globin RNA only in the presence of tat 1 would prove trans-activation. (3) transfection of erythroid cells by the tat 1 expression vector will allow study of trans-activation of globin genes within the milieu of the intact erythroid cell. Other regulatory factors, such as chromatin structure, methylation, and the actual erythroid trans-factors will thus be in a normal state.

If the initial experiments demonstrate trans-activation, more detailed questions will be asked, such as which DNA sequences are involved in the interaction and whether trans-factors activate or repress particular genes. The ultimate goal is to facilitate identification of the factors regulating globin expression in normal erythroid cells. (Fox, Schechter)

H. Oncogenes and the Control of Globin Gene Expression

Humans undergo two developmental switches in their hemoglobin phenotype, the embryonic to fetal switch occuring in early gestation and the fetal to adult switch occuring around the time of birth. Gene regulation has two main components, namely cis-acting DNA sequences and trans-acting molecules (presumably proteins that interact with DNA sequences in a specific manner to control gene expression. The K562 human leukemia cell line expresses all globin genes other than the adult beta-globin. Previous work from this laboratory showed that the beta-globin gene of K562 cell functions normally in a heterologous expression system. Elucidation of the mechanism of failure of beta-globin gene expression in K562 cells may provide an insight into globin gene expression and switching in normal erythroid cells.

The human adenovirus E1a protein products in trans on the transcription of other viral genes as well as certain resident cellular genes such as hsp 70. An enhancer deficient human beta-globin gene is not transcribed in HeLa cells; however, cotransfection with the E1a gene results in easily detectable beta-globin mRNA. The c-myc protein nuclear matrix product has limited homology to adenovirus E1a protein and like E1a can induce expression from the hsp 70 promoter and can complement c-ras in transforming primary cells. We propose to transfect a hybrid myc plasmid with a dexamethasone responsive regulatory element (MMTV-Xba-myc) into K562 cells and study epsilon and beta-globin gene expression upon steroid induction. Hybrid epsilon- and beta-CAT plasmids will be cotransfected with MMTV-Xba-myc and the level of CAT activity assayed in heterlogous systems. No suitable human erythroid cell lines expressing only beta-globin are available. Such line(s) will be established using MMTV-Xba-myc and a c-ras containing oncogene. The steroid inducible MMTV element should permit mimicry of the in vivo decrease in c-myc mRNA level seen with differentiation, as well as supply an adult hemoglobin forming cell line. (Dave, Schechter)

I. Laboratory and Clinical Models for the Study of Globin Gene Expression

We are investigating the molecular mechanisms which control the individual and total concentrations of hemoglobins in human erythrocytes. In addition, we are studying the effects of functional alpha globin gene number, fetal hemoglobin (HbF) levels and the extent of red cell heterogeneity on the various manifestations of sickle cell disease and its genetic variants. The levels of each of the normal hemoglobins (A, A2, F) are determined by controls at the level of transcription and/or translation of the globin genes, as well as by factors that regulate protein degradation. The study of the control of hemoglobin levels has direct relevance to various hemoglobinopathies especially thalassemia and sickle cell disease. In addition, these studies are of potential relevance to the more general question of control of gene expression in eukaryotic cells. For our experimental system, we are using the K562 human leukemic cell line, as well as peripheral blood from individuals with sickle cell disease. We are studying the effects of short-term and long-term exposure of these cells to 5-azacytidine on their phenotype and the factors that control globin gene transcription. Concurrently, we are also attempting to develop a sickle cell mouse model by the introduction of a cloned human sickle globin gene into the mouse germ line by the microinjection of DNA into the pronuclei of fertilized eggs. The establishment of such a model would allow for basic and fundmental questions to be asked

about the molecular, cellular and physiologic aspects of the disease, as well as provide an in vivo system to monitor the effects of potential therapy. (Rodgers,Noguchi, Ahmed, Schechter)

J. Factors Affecting Mouse Beta-Globin Gene Expression

In order to analyze the sequence requirements for induction of the mouse beta(maj)-globin gene, we have developed a transient assay system in mouse erythroleukemia (MEL) cells. These cells, which have been transformed by the Friend virus complex, can be chemically induced to undergo terminal differentiation during which transcription of endogenous alpha and beta globin genes is greatly increased. If we can mimic this effect in a transient assay, which only requires 4 to 5 days, then it should be possible to quickly and accurately analyze plasmid constructions with varying amounts of DNA 5' or 3' to the beta-globin promoter to determine what regions are required for induction. Transient assay conditions have been optimized for both uninduced and induced MEL cells and we currently wish to determine if induction affects genes located on transfected plasmids which in these experiments remain episomal.

We have previously shown that DNA sequences known as enhancers increase the activity of the mouse beta-globin promoter in transient assays. Enhancers are cis-acting DNA sequences which act at the level of transcription to increase gene expression. They can function in either orientation both 3' and 5' to the target gene and their level of activation is relatively independent of position. While there is no high degree of sequence homology among the presently identified enhancers, two categories of short "core" regions have beeen observed. We are interested in determining if any other common features of enhancer DNA sequences exist and, if so, whether they might suggest possible mechanisms of enhancer activation of the mouse beta-globin promoter as well as other enhancer activated promoters. Analysis of five enhancers has shown that each exhibits dyad symmetry; we are extending this analysis to include additional enhancers. We have also shown that known enhancer mutants exhibit less dyad symmetry than the wild type enhancer, suggesting there may be a correlation between enhancer function and degree of dyad symmetry. (Berg-Lovett, Schechter)

II. Molecular, Cellular, and Clinical Pathophysiology of Sickle Cell Disease

A. Sickle Cell Anemia: The Intracellular Polymerization of Hemoglobin S

We have demonstrated that the extent of intracellular polymerization of hemoglobin S is primarily determined by oxygen saturation, hemoglobin concentration and hemoglobin composition. Polymer can be detected in sickle erythrocytes at high oxygen saturation values and accounts for most of the variation among sickle syndromes. Cell heterogeneity can modify polymer formation and an increase in erythrocyte density occurs in sickle cell anemia. Homozygous alpha-thalassemia in sickle cell patients decreases cell heterogeneity and hemolysis, but the small decrease in polymerization is not sufficient to give the dramatic improvement associated with sickle cell disease with HPFH or sickle trait. Our recent direct measurements of cell filterability indicate that the polymer formation at high oxygen saturation does affect cell rheology, particularly in the dense cells. Our calculations of polymer formation can be used to provide goals for major therapeutic approaches, with respect to the amount of polymer reduction necessary to achieve various levels of clinical improvement.

The K562 human continuous cell provides a model system for examining the factors which determine the concentration and composition of globin produced within erythroid cells. In order to minimize the heterogeneity of K562 cells in response to hemin induction, K562 cells have been grown in the presence of hemin for long term (22 months). These cells are being characterized and compared with uninduced and short-term induced (4 to 7 days) K562 cells, for intracellular and surface factors which relate to hemoglobin production.

The quantitative relationship between hemoglobin S polymerization and pathophysiology remains unclear. We have begun to develop transgenic mice with sickle cell anemia by inserting human sickle beta and alpha genes as a potential animal model for studying the physiological effects associated with hemoglobin S polymerization and abnormal cell rheology. (Noguchi, Rodgers, Poillon, Torain, Schechter)

B. The Development of Non-Invasive Methods to Assess Sickle Cell Patients

Despite recent biophysical insights into the molecular pathogenesis of the sickle cell syndromes, our understanding of the relationship of these subcellular events to the variable clinical expression of sickle cell disease remains largely speculative. We have sought to develop quantitative ways to clarify disease pathogenesis, as well as to assess severity and progression. We have developed an analytical phthalate ester technique for separating sickle erythrocytes by density (or intracellular hemoglobin concentration) and have calibrated this method against the preparative Stractan method. Using the phthalate method we have now shown that there are at least three cellular processes contributing to red cell heterogeneity and we are investigating the genetic and biochemical processes that account for the appearance of dense cells in individuals with sickle cell anemia. Ocular studies of the patients show striking correlations between the extent of erythrocyte heterogeneity with conjunctival and retinal pathology. In other physiological studies, we have been evaluating the utility of several non-invasive approaches which characterize the consequences of altered microvascular blood flow. Using the technique of laser-Doppler velocimetry, we have found that forearm cutaneous microcirculatory flow undergoes a unique characteristic periodic pattern. Studies of 2-deoxy-18 fluoro-deoxyglucose brain metabolism using positron emission tomography (PET) have demonstrated frequent anterior cerebral pathology where none was previously suspected. Preliminary studies using magnetic resonance imaging (MRI) suggests that this modality is more sensitive than conventional methods in disclosing early changes of aseptic necrosis of the femoral heads. We hope that these cellular and physiological measurements will allow us to understand better the extreme spectrum of disease manifestations, as well as serve as objective means of evaluating response to therapy in sickle cell anemia patients. (Rodgers, Noguchi, Schechter)

III. Molecular Biology of Human Lymphoid Cells

A. Human T Cell Receptor Genes: Beyond Alpha and Beta

Important progress has been made in understanding the process by which T lymphocytes recognize antigen through the cloning and characterization of the alpha and beta genes of the T cell receptor for antigen (TCR). Despite this progress, the mechanism by which T lymphocytes recognize antigen in association with a product of the major histocompatibility locus (MHC), termed dual recognition, remains to be elucidated. This process has particular importance to our

understanding allograft rejection and tolerance, which is critical for improving the success of renal and bone marrow transplants. It is becoming increasingly likely that understanding the expression of the alpha and beta genes alone will not explain dual recognition, and recent data from several laboratories suggest that additional TCR genes are involved. One such putative gene, termed gamma, has been isolated but remains poorly characterized. This project aims to define additional human T cell receptor genes with the goal of better understanding the process of dual recognition.

We have begun searching for additional human TCR genes which are postulated, like gamma, to participate in dual recognition. The initial approach has been to cross-hybridize a highly conserved region of mouse TCR genes selected by computer analysis with human genomic clones. This strategy, when applied previously by other laboratories for screening MHC genes, was useful in identifying new, unsuspected MHC genes. We have obtained a genomic clone, SLE1, which cross hybridizes with our murine TCR probe but is not the known human alpha, beta, or gamma genes. We are in the process of characterizing this clone to determine if it is a true TCR gene, but similar to authentic TCR genes, we have seen rearrangement of this gene in the DNA derived from some human T cell tumors. Subsequent studies will also employ antigen-specific human suppressor cells, which do not transcribe the beta TCR gene, and so must be using additional genes for antigen recognition. (Cohen, deVillartay, Nielsen)

B. Molecular Analysis of Self-Education

The process of self-education selects appropriate antigen-specific T cell receptors (TCRs) which are tolerant to determinants of the major histocompatibility locus (MHC) alone, but which can interact with self-MHC in the presence of antigen.

Recently the TCR has been characterized at the molecular level. The TCR is constituted by the association of at least two polypeptide chains, alpha and beta, which contribute to the specificity of the T cell. These two polypeptides are encoded by two distinct sets of genes. In addition to these two subunits, a third set of genes encoding a putative TCR molecule, called gamma chain, has been found expressed at the mRNA level in certain populations of T cells. Nevertheless, the protein product of this gene as well its function are not known to date. The genes encoding these three polypeptides are present in an unrearranged form in germline DNA and undergo specific rearrangement in DNA of cells of the T cell lineage.

The pattern of these rearrangements presumably controls the specificity and tolerance of the T cells. We have described a situation in humans following MHC haploidentical Bone Marrow Transplantation (BMT) in which tolerance to donor MHC determinants did not occur, leading to the circulation of donor T cells displaying reactivity towards donor histocompatibility determinants. An autoreactive T cell line from this patient has been grown in vitro. We propose to study the expression of the TCR genes in this autoreactive cell line. The same study will also be done in mice in which it has been possible to induce severe auto-Graft-versus-Host (GVH) reactions in vivo after syngeneic BMT. The study of TCR expression in these two abnormal situations should give us some insight into the process of normal self-education at the molecular level, and should contribute to transplantation therapy in general. (deVillartay, Cohen)

C. Survey of Human Lymphoid Diseases for Human Pathogenic Retroviruses

Retroviruses have been postulated or established to play an etiologic role in many human lymphoid malignancies as well as in certain human autoimmune diseases. Despite this hypothesis, actual proof implicating human retroviruses in most of these diseases has remained elusive. Nevertheless, the recent isolation of two pathogenic retroviruses (HTLV I and II) from human T cell malignancies and the characterization of a third retrovirus (HIV) as the causative agent of acquired immunodeficiency disease (AIDS) has reawakened interest in this model. In particular, new data concerning the life cycle and growth of HIV have suggested new approaches to this problem. My laboratory has familiarized itself with these approaches by studying retroviral gene expression in several autoimmune mouse strains, and HIV. Now we are collecting human tissue with the goal of identifying new human pathogenic retroviruses.

To date, we have assayed DNA and RNA from patient with juvenile rheumatoid arthritis, rheumatoid arthritis, and systemic lupus for evidence of pathogenic retroviruses. We found that they do not contain sequences capable of cross-hybridizing with probes derived from HTLVI or HIV. In addition, the RNA did not contain one form of human endogenous retrovirus at elevated levels. While these studies probably rule out these, or closely related retroviruses, as causing these diseases, they do not invalidate the general model. Each new type of human pathogenic retrovirus has been distinctly different from previous isolates. The discovery of a pathogenic retrovirus in any of these diseases would be a dramatic breakthrough in understanding its etiology, and might establish a mechanism of preventing that disease through vaccination. (Cohen, deVillartay)

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 25008-23 LCB
formerly
Z01 AM 25008-22 LCB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Chemical Synthesis of Cytochrome c: The Roles of Individual Residues

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Hiroshi Taniuchi,	Chief, Section on Protein Chemistry and Conformation	LCB, NIDDK
Other:	Luigia Gozzini	Guest Researcher	LCB, NIDDK
	Yu-cang Du	Guest Researcher	LCB, NIDDK
	Alice Fisher	Chemist	LCB, NIDDK

COOPERATING UNITS *(if any)*
University of Padova, Padova, Italy

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SECTION
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INSTITUTE AND LOCATION
NIDDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	1.3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The mitochondrial cytochromes c of the present species are presumed to collectively reflect evolutionary events occurring for the last 1.5 billion years. These evolutionary secrets, if uncovered through finding the roles of the invariant residues, may help understanding the principles underlying structure-function of proteins. In this context, it would be worthwhile to chemically synthesize the ancestral form of cytochrome c predicted by W.M. Fitch and E. Margoliash and test whether it is active. The feasibility of this project is supported by our finding that the complementing fragments are completely exchangeable between horse and Candida krusei cytochromes c for formation of hybrid complexes (see the other report). As a first step, we plan to synthesize horse cytochrome c via synthesis of apocytochrome c, using the Merrifield solid phase method and joining two apofragments (complexation assisted rejoining, see the previous report), followed enzymatic attachment of heme, using cytochrome c synthase found in this Section. In extending the joining procedure we have shown that apofragment [Hse-lactone-65](23-65) (homoserine-lactone), but not [Hse-lactone-65](39-65), of horse cytochrome c rejoins to apofragment (66-104) in the presence of reduced heme fragment (1-25)H and that intact methionine 80 is essential for this rejoining reaction. On the basis of these results, to make semi-synthetic [Hse-65](28-104), we carried out synthesis of fragment [Gly-66](28-66) of horse cytochrome c, containing tryptophan at position 59.

In the effort to develop a second procedure for joining of fragments, we found a novel carboxypeptidase A catalyzed transpeptidation as follows. Synthesized radiolabeled tripeptide Leu-Met-His-amide or Leu-Arg-Met-amide and excess of heme fragment [Hse-69](1-69)H of yeast cytochrome c or [Hse-65](1-65)H of horse cytochrome c were incubated with carboxypeptidase A in the presence of 50 to 70% glycerol at pH 10.0 at 20 to 30°C. Analyses of the product indicated that both labeled tripeptides replaced the carboxyterminal homoserine with the efficiency up to 50% after 7 days.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 25011-12 LCB
formerly
Z01 AM 25011-11 LCB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Mechanism of Protein Folding: Global Coupling - A New Type of Interaction

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Hiroshi Taniuchi	Chief, Section on Protein Chemistry and Conformation	LCB, NIDDK
Others:	Marek Lisowski	Visiting Fellow	LCB, NIDDK
	Alice Fisher	Chemist	LCB, NIDDK
	Xuan Truong	Biological Aid	LCB, NIDDK

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2.8	2.4	0.4

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The studies of the fragment complexes of staphylococcal nuclease, RNase A and cytochrome c have led us to the hypothesis that after folding of almost the entire polypeptide chain (including the S-S bonds in the case of RNase A) the interatomic interactions would be globally coupled to generate extra force for shifting the equilibrium of folding and unfolding in favor of folding. To understand this extra force and speculating that some of evolutionarily invariant amino acids might play a role we have investigated the effect of substitution of invariant proline 30, leucine 32 and glycine 34 and partially invariant Leu 35 of cytochrome c using the three-fragment complex of horse cytochrome c as described in the previous years. The first phase of this work is now complete with important results: (1) The extra force would not be van der Waals interaction, hydrogen bond, hydrophobic interaction or electrostatic interaction per se, i.e. this force would be a new type of interaction having a property of delocalization; (2) The extra force would constrain the atomic positions of individual residues in a concerted manner throughout the structure; (3) The extra force would be detectable on the basis of perturbation of enthalpy and entropy changes (the same sign) associated with folding by substitution of some specific amino acid such as evolutinarily invariant one after taking into account the contributions of possible perturbation of the unfolded form. In the case of cytochrome c the extra force would be stronger for the reduced form than for the oxidized form thus modulating the redox potential. Further, the studies of hybrid complexes using fragments from horse, tuna, Candida krusei, and yeast cytochromes c have indicated that the information for the cytochrome c fold is exchangeable between the fragments of phylogenetically distant species as measured by ligation of Met 80 to the heme iron, and suggested that; (a) however, mutation of a few specific amino acids or perhaps a single amino acid could alter stabilization of the Met 80-S-heme-Fe bond; (b) such destabilization could be reversed by mutation at some other position or positions.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 25,016-13 LCB
formerly
Z01 DK 25,016-12 LCB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*
Factor(s) Controlling Globin Gene Expression in K562 Cells.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principel Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Jacques Elion	Visiting Fellow	LCB, NIDDK
Others:	Pat Berg	Senior Staff Fellow	LCB, NIDDK
	Shi-Xian Cao	Visiting Fellow	LCB, NIDDK
	Alan N. Schechter	Chief	LCB, NIDDK

COOPERATING UNITS *(if any)*

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	0

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☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

K562 cells are used as a model to study the control of human globin gene expression. They express embryonic and fetal but not adult globin genes. Results suggest that the lack of transcription of the adult beta-globin gene results from an alteration of a regulating trans-acting factors(s). Our objectives are to provide evidence for the existence of such a factor, to elucidate its nature as an activator or a repressor and to set up an in vitro assay for its measurement. Globin promoters activity is deduced from the transient expression of the bacterial CAT enzyme in K562 cells transfected with plasmids in which transcription of the CAT gene is driven by various globin gene promoters. In competition experiments, cells have been cotransfected with these plasmids and an excess of the corresponding globin promoter. However, low efficiency and poor reproducibility of transfection impaired analysis of the results. Therefore, details of the transfection procedure have been thoroughly worked out to optimize reproducibility and efficiency. Since competition experiments required final DNA concentrations found to be inhibitory of CAT expression in control experiments, we are now constructing competitor plasmids containing multiple copies of the globin promoter sequences. Experiments conducted with K562 cells grown in the presence of 5-azacytidine, did not provide evidence for expression of the CAT plasmid containing the beta-promoter, but showed non-specific enhancement of expression of the other CAT plasmids. Finally, we are attempting to develop an assay for trans-acting factors that would allow us to track them in a purification scheme. We constructed SP6 promoter-containing plasmids in which the globin promoters are used as templates for in vitro transcription. We hope to be able to set up conditions in which binding of proteins to the promoter will result in premature transcription termination. Labelled transcripts could then be analyzed by polyacrylamide gel electrophoresis and autoradiography.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 25021-11 LCB
formerly
Z01 AM 25021-10 LCB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*
Sickle Cell Anemia: The Intracellular Polymerization of Hemoglobin S

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	Constance Tom Noguchi	Research Physicist	LCB, NIDDK
Others:	Griffin P. Rodgers	Guest Researcher	LCB, NIDDK
	William Poillon	Guest Researcher	LCB, NIDDK
	Barbara Torain	Biological Lab Technician	LCB, NIDDK
	Alan N. Schechter	Chief	LCB, NIDDK

COOPERATING UNITS *(if any)*
MET, NCI (T. Waldmann); LMG, NICHD (H. Westphal); Lawrence Natl. Lab., (R.H. Jensen); Johns Hopkins, (G. Dover); Jamaica (G. Serjeant); Birmingham, U.K. (J. Stuart); Cambridge, U.K. (M. Perutz); Oxford, U.K. (D.J. Weatherall, D.R. Higgs).

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2.3	1.8	0.5

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☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have demonstrated that the extent of intracellular polymerization of hemoglobin S is primarily determined by oxygen saturation, hemoglobin concentration and hemoglobin composition. Polymer can be detected in sickle erythrocytes at high oxygen saturation values and accounts for most of the variation among sickle syndromes. Cell heterogeneity can modify polymer formation and an increase in erythrocyte density occurs in sickle cell anemia. Homozygous alpha-thalassemia in sickle cell patients decreases cell heterogeneity and hemolysis, but the small decrease in polymerization is not sufficient to give the dramatic improvement associated with sickle cell disease with HPFH or sickle trait. Our recent direct measurements of cell filterability indicate that the polymer formation at high oxygen saturation does affect cell rheology, particularly in the dense cells. Our calculations of polymer formation can be used to provide goals for major therapeutic approaches, with respect to the amount of polymer reduction necessary to achieve various levels of clinical improvement.

The K562 human continuous cell provides a model system for examining the factors which determine the concentration and composition of globin produced within erythroid cells. In order to minimize the heterogeneity of K562 cells in response to hemin induction, K562 cells have been grown in the presence of hemin for long term (22 months). These cells are being characterized and compared with uninduced and short-term induced (4 to 7 days) K562 cells, for intracellular and surface factors which relate to hemoglobin production.

The quantitative relationship between hemoglobin S polymerization and pathophysiology remains unclear. We have begun to develop transgenic mice with sickle cell anemia by inserting human sickle beta and alpha genes as a potential animal model for studying the physiological effects associated with hemoglobin S polymerization and abnormal cell rheology.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 25025-10 LCB
formerly
Z01 AM 25025-09 LCB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Origin of the specificity of antigen-antibody interaction

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Hiroshi Taniuchi, Chief, Section on Protein Chemistry and Conformation		LCB, NIDDK
Others:	Ida Silvestri	Visiting Fellow	LCB, NIDDK
	Alice Fisher	Chemist	LCB, NIDDK

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NIDDK

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The studies in this Section have led us to the hypothesis that the energy state of one region of the structure of proteins would be coupled with those of distant regions to generate extra stabilizing energy. Thus, to investigate whether the strength of antigen binding is related to the reduction of the fluctuation of distant regions of the antibody by such coupling, we have developed 5 hybridoma cell lines, 4.74.6, 4.128.6, 4.145.10, 2.96.12 and 2.34.19 producing monoclonal IgG1 directed against yeast iso-1-cytochrome c. Monoclonal IgG1 4.74.6, 4.128.6 or 4.145.10 reacts with yeast cytochrome c but not with cytochrome c of Candida krusei, tuna, chicken, pigeon, rat, sheep, dog porcine, bovine or horse. Monoclonal IgG1 2.96.12 or 2.34.19 reacts with both yeast and C. krusei cytochromes c. The former IgG1, but not the latter also reacts with tuna, rabbit and chicken cytochromes c in a lesser extent. None of these five monoclonal antibodies reacts with the apocytochromes c tested (yeast, C. krusei and horse). Monoclonal IgG1 4.74.6, 4.128.6 or 4.145.10 does not react with heme- or apofragments of yeast cytochrome c but does react with the productive fragment complexes of yeast cytochrome c or even with the hybrid complexes containing the horse heme fragment and yeast apocytochrome c. On the basis of tests with hybrid complexes, the antigenic determinants for these three antibodies appear to be located in residues 59 to 108. Monoclonal IgG1 2.96.12 and 2.34.19 do not react with any of these complexes or hybrid complexes. The results indicate that all of these five monoclonal antibodies recognize the three-dimensional configuration of the antigenic determinant formed by folding of the native cytochrome c (and also the complexes in the three former cases). In addition, the three former antibodies more specifically recognize the side chains of the amino acids in the determinant than the two latter. However, the two latter antibodies discriminate between the native protein and the fragment complex but the three former do not.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 25028-08 LCB
formerly
Z01 AM 25028-07 LCB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Development of Non-Invasive Methods to Assess Sickle Cell Patients

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Griffin P. Rodgers	Guest Researcher	LCB, NIDDK
Others:	Constance T. Noguchi	Senior Investigator	LCB, NIDDK
	Alan N. Schechter	Chief	LCB, NIDDK

COOPERATING UNITS *(if any)*
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SECTION
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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

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☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Despite recent biophysical insights into the molecular pathogenesis of the sickle cell syndromes, our understanding of the relationship of these subcellular events to the variable clinical expression of sickle cell disease remains largely speculative. We have sought to develop quantitative ways to clarify disease pathogenesis, as well as to assess severity and progression. We have developed an analytical phthalate ester technique for separating sickle erythrocytes by density (or intracellular hemoglobin concentration) and have calibrated this method against the preparative Stractan method. Using the phthalate method we have now shown that there are at least three cellular processes contributing to red cell heterogeneity and we are investigating the genetic and biochemical processes that account for the appearance of dense cells in individuals with sickle cell anemia. Ocular studies of the patients show striking correlations between the extent of erythrocyte heterogeneity with conjunctival and retinal pathology. In other physiological studies, we have been evaluating the utility of several non-invasive approaches which characterize the consequences of altered microvascular blood flow. Using the technique of laser-Doppler velocimetry, we have found that forearm cutaneous microcirculatory flow undergoes a unique characteristic periodic pattern. Studies of 2-deoxy-18 fluoro-deoxyglucose brain metabolism using positron emission tomography (PET) have demonstrated frequent anterior cerebral pathology where none was previously suspected. Preliminary studies using magnetic resonance imaging (MRI) suggests that this modality is more sensitive than conventional methods in disclosing early changes of aseptic necrosis of the femoral heads. We hope that these cellular and physiological measurements will allow us to understand better the extreme spectrum of disease manifestations, as well as serve as objective means of evaluating response to therapy in sickle cell anemia patients.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 25038-06 LCB
formerly
Z01 AM 25038-05 LCB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Effects of HTLV-1 Tat Gene Product on Globin Expression

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Henry B. Fox	NRSA Fellow	LCB, NIDDK
Others:	Alan N. Schechter	Chief	LCB, NIDDK

COOPERATING UNITS *(if any)*
Metabolism Branch, NCI (Drs. T. Waldmann and W. Greene); LTCB, NCI (Drs. H. Streicher and R. Gallo)

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Laboratory of Chemical Biology

SECTION
Section on Molecular Biology and Genetics

INSTITUTE AND LOCATION
NIDDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The control of human globin gene expression in erythroid cells involves trans-factors (substances active at multiple locations in the genome), which have yet to be identified or clearly described. One experimental approach to their identification is to study the effects on globin gene expression of well-described trans-factors from tumor viruses. Trans-factors from adenovirus 5 and pseudorabies virus are known to stimulate beta-globin transcription. HTLV-I specifies a similar trans-factor, tat 1, whose effect on non-viral genes is not known. We have designed three lines of transfection experiments to test the interaction of tat 1 and globin genes: (1) a normally inactive beta-globin plasmid is transfected into C81-66-45 cells, which produce tat 1. Detection of beta-globin RNA would suggest trans-activation of this gene by tat 1. (2) nonerythroid cells are cotransfected by a tat 1 expression vector and a beta-globin plasmid. Expression of beta-globin RNA only in the presence of tat 1 would prove trans-activation. (3) transfection of erythroid cells by the tat 1 expression vector will allow study of trans-activation of globin genes within the milieu of the intact erythroid cell. Other regulatory factors, such as chromatin structure, methylation, and the actual erythroid trans-factors will thus be in a normal state.

If the initial experiments demonstrate trans-activation, more detailed questions will be asked, such as which DNA sequences are involved in the interaction and whether trans-factors activate or repress particular genes. The ultimate goal is to facilitate identification of the factors regulating globin expression in normal erythroid cells.

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 25042-05 LCB

PERIOD COVERED

Terminated as of September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Sickle Cell Disease: The Rheological Effects of Intracellular Polymerization

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Alan N. Schechter	Chief	LCB, NIDDK
Others:	Clemenceau Acquaye	Visiting Fellow	LCB, NIDDK

COOPERATING UNITS *(if any)*

BEIB (E.L. Walker); LCDB, NIDDK (Dr. E.J. Blanchette-Mackie).

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated.

GPO 904 917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 25045-03 LCB
formerly
Z01 AM 25045-02 LCB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regulation of Globin Gene Expression by 5' DNA Sequences

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Patricia Berg-Lovett	Senior Staff Fellow	LCB, NIDDK
Others:	Yu Gong	Visiting Fellow	LCB, NIDDK
	Jacques Elion	Visiting Fellow	LCB, NIDDK
	Ruo-Lan Qian	Guest Researcher	LCB, NIDDK
	Alan N. Schechter	Chief	LCB, NIDDK

COOPERATING UNITS *(if any)*
Laboratory of Molecular Hematology, NHLBI (Dr. D. Williams); Experimental Hematology Division, AFRRI (Dr. W.D. Hankins)

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INSTITUTE AND LOCATION
NIDDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.3	2.3	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In order to better understand the switching of human globin synthesis from embryonic to fetal to adult globin, we are studying a human cell line, K562, blocked in the transition from fetal to adult globin synthesis. K562 cells are unable to synthesize beta-globin mRNA. Previous studies from this laboratory have shown the molecular defect in K562 cells appears to be in a trans-acting factor. The most likely possibilities are that a repressor is continuously synthesized which binds to DNA near the beta-globin promoter, preventing transcription, or that an activator which is necessary for beta-globin transcription is not synthesized, or that K562 cells both synthesize a repressor and lack an activator.

To test these hypotheses we are using a plasmid, p-beta-GLCAT, which contains the human beta-globin promoter and additional 5' DNA fused to an easily assayable gene, CAT (chloramphenicol acetyl transferase). The fusion gene, beta-CAT, is inactive in K562 cells so we are attempting to activate it using several approaches. Our experiments to activate beta-CAT either in cis, with the SV40 enhancer, or in trans, with the E1A protein, have been unsuccessful in K562 cells but successful in Chinese hamster cells, consistent with the presence of a repressor in K562 cells. Further support for this model comes from 5' deletion mutants of beta-CAT, which indicate that deletion of certain 5' sequences does allow beta-CAT expression in K562 cells. Thus, there seems to be a negative regulatory region near the beta-globin promoter. Experiments are underway to try to identify the DNA binding site of the putative repressor and to identify the repressor molecule itself.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 25046-02 LCB
formerly
Z01 AM 25046-01 LCB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Control of Exocytosis in Sea Urchin Eggs by Osmotic Stress

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Joshua Zimmerberg	Guest Researcher	LCB, NIDDK
Others:	V.A. Parsegian	Chief, Section on Molecular Forces and Assembly	LCB, NIDDK

COOPERATING UNITS *(if any)*
University of London, UK (Dr. M. Whitaker); Harvard University, Cambridge, MA (Dr. J. Liu).

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NIDDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have continued to examine the role of osmotic swelling of secretory granules during exocytosis. This year, we are focusing on exactly how this swelling is accomplished. We examine sea urchin egg cortical granule exocytosis with differential interference contrast light microscopy, phase contrast microscopy, fluorescence microscopy, and intracellular recording to measure cell capacitance and potential. Compromising the integrity of the secretory granule membrane to the extent of allowing free passage of small molecules does not alter calcium stimulated secretion in vitro. Exocytosis proceeds without ions. Granules remain intact in the presence of concentrations of digitonin sufficient to cause lucifer yellow entry. Higher concentrations of digitonin cause the same phase-transition of contents seen with calcium. This phase transition can be inhibited reversibly with osmotic stress. Exocytosis is prevented by including a variety of polymers of different chemical composition and molecular weight in the sea water surrounding the eggs. The increase in membrane capacitance which occurs during exocytosis is not greatly altered by inhibitory concentrations of polymer. Calcium and magnesium reduce the osmolality required to prevent exocytosis. These results suggest that calcium causes swelling by an alteration in the state of the internal granule phase to increase the affinity of this phase for water rather than by inducing ionic fluxes into the granule.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 25047-02 LCB
formerly
Z01 AM 25047-01 LCB

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 cherecters or less. Title must fit on one line between the borders.)*

Hydration Forces and Applications of the Osmotic Stress Technique

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Donald C. Rau	Senior Staff Fellow	LCB, NIDDK
Others:	V. Adrian Parsegian	Chief, Section on Molecular Forces and Assembly	LCB, NIDDK

COOPERATING UNITS *(if any)*

Dr. Thomas M. Jovin, Molecular Biology Department, Max Planck Institute for Biophysical Chemistry, Gottingen, West Germany.

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NIDDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.9	0.9	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Hydration forces are the recently uncovered interactions between DNA or lipid bilayer surfaces that dominate the energies between these surfaces at separation distances of 20 angstroms and less. These forces appear to be due to the structuring of water between surfaces and can be either strongly attractive or strongly repulsive depending on the surface hydration. We can directly measure these forces by combining the osmotic stress technique with x-ray diffraction to measure the separation of the surfaces. In analyzing the energetics of these forces, we have concentrated on the osmotic pressure induced assembly of Mn2+-DNA. The force curves show an abrupt transition at a critical osmotic pressure, that depends on temperature and Mn2+ concentration, between repulsive and attractive hydration forces, mediated by the presumed rearrangement on Mn2+ on the surface of DNA. The transition is entropically driven presumably by the release of bound water. We have now examined the effect on the transition of different anions that structure bulk water differently. The transition occurs more readily with ClO4- than with Cl- and is more difficult with SO4/2^{-} than with Cl-. We have quantitated the entropy changes and found that the differences are due to the additional entropy gained or lost by releasing structured water around DNA helices into the bulk. This is the first clear, direct indication that water structuring is the source of hydration forces.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 25048-02 LCB
formerly
Z01 AM 25048-01 LCB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular forces

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: V.A. Parsegian Chief, Section on Molecular Forces and Assembly LCB, NIDDK

COOPERATING UNITS *(if any)*
R.P. Rand, Brock University, Canada; E.A. Evans, Univ. British Columbia, Canada; S.M. Gruner, Princeton Univ.; E. Barouch, Clarkson Univ.

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NIDDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The PI, Sol Gruner and Peter Rand have succeeded in determining the mechanical properties of a non-lamellar lipid phase, the inverted hexagonal form thought to correspond to transient structures during membrane fusion. It appears that different combinations of lipids have different most-favored or "intrinsic" radii of curvature. The work of forced deviations from this configuration can be described in terms of a bending modulus whose value is nearly the same for all lipids. It also seems that in these non-lamellar forms the hydrocarbon chains are under little strain but rather act to fill space. Hydrocarbons added to bilayers will tend to drive the lipids to non lamellar forms.

These lines of physical reasoning lead immediately to better understanding of the reasons for the biochemical metabolic steps in lipid metabolism. It also provides a strong indication for a morphological role for hydrocarbons such as dolichol known for its biochemical function anchoring oligosaccharides during synthesis.

With Evan Evans and Donald Rau, the PI has further pursued the problem of relating the mechanical motion of neighboring membranes or linear macromolecules to the long-range forces acting between them. We have used a measure of extent of molecular motion from the widths of x-ray diffraction peaks scattering from arrays of condensed DNA together with direct force measurements to see how forces and motion affect each other.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 25049-02 LCB
formerly
Z01 AM 25049-01 LCB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Chromatin Structure and Trans-activation of Human Globin Genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Shi Xian Cao	Visiting Fellow	LCB, NIDDK
Other:	Jacques Elion	Visiting Fellow	LCB, NIDDK
	Helena Mishoe	Staff Fellow	LCB, NIDDK
	Alan N. Schechter	Chief	LCB, NIDDK

COOPERATING UNITS *(if any)*

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INSTITUTE AND LOCATION
NIDDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Chromatin structure and trans-activation are two important aspects of gene regulation. Chromatin from actively transcribed genes exhibits a structure different from that of non-transcribed genes. It has been shown that trans-acting proteins can bind to DNA and confer a unique chromatin structure, and such a protein DNA interaction has been suggested to play an important role in gene regulation. In an attempt to investigate the mechanisms of regulation of human globin gene expression two approaches have been used: 1) We have studied the chromatin structure around the human beta-globin gene in K562 cells by using DNase I and S1 nuclease. Despite the beta-globin gene not being expressed, we found nuclease hypersensitive sites in both its 5' and 3' flanking regions. While the 3' hypersensitive sites are identical to that of active beta globin genes, the 5' hypersensitive sites shift further upstream. This suggests that the lack of beta-globin expression in K562 cells may be related to unique aspect(s) of the chromatin structure in the immediate 5' flanking region of the beta-globin gene. 2) To investigate the mechanism of trans-activation, globin promoters were tested in a transient expression system. We found that the globin promoter can be activated by SV40 T antigen in monkey kidney CV-1 cells. This trans-activation can be further stimulated by including a SV40 enhancer in the globin plasmid. The mechanism of the trans-activation is currently under study.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 25050-02 LCB
formerly
Z01 AM 25050-01 LCB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structure and Physical Properties of DNA and DNA-Protein Complexes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Donald C. Rau Senior Staff Fellow LCB, NIDDK

COOPERATING UNITS *(if any)*
H.-H. Chen, George Mason University, Fairfax, Virginia; A. Maxwell, M. Gellert and J. Nickol, LMB, NIDDK

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NIDDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Progress has been made in physically characterizing three distinct systems, all of which have possible biological significance. A substantial difference in the bending energy or flexibility has been found between poly (dG-dC) and its methylated analogue, poly (dG-m5dC). This difference makes it probable that resistance to bending is largely due to perturbations in groove hydration rather than in base stacking interactions. It also suggests that a biological effect of CpG methylation is to potentiate the ease of nucleosome formation relative to protein factor binding.

We have identified the source of entropy, that drives the conformational transition from the B to Z forms of poly(dG-m5dC), as the release of two water molecules per base pair. This result suggests that this transition will be significantly easier in the cell, under conditions of osmotic stress, than in dilute solution.

The conformational change that occurs with ATP binding to gyrase-DNA complexes has been determined. The DNA tails, that extend out from the core in the basic complex, fold back cross the protein when ATP is added. These results will enable us to correlate mechanism, biochemistry, and structure for the supercoiling reaction of gyrase.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 25051-02 LCB
formerly
Z01 AM 25051-01 LCB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Transcription of Globin Genes In Vitro

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigetor.) (Name, title, laboratory, and institute affiliation)*

PI:	Helena Mishoe	Staff Fellow	LCB, NIDDK
Others:	Alan N. Schechter	Chief	LCB, NIDDK

COOPERATING UNITS *(if any)*

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NIDDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The K562 leukemic cell line can be used as a model system to increase our understanding of events associated with developmental expression and regulation in normal marrow progenitor cells.

We are working to establish an in vitro transcription system in which the transcription pattern of the globin genes mimick that observed in vivo, i.e. expression of embryonic and fetal, but not adult globins. As an initial approach, in vitro transcriptional analysis using a whole cell and nuclear extract made from K562 cells was carried out. We have demonstrated that all cloned globin genes (beta, epsilon, and gamma) are transcribed in vitro indicating a lack of differential transcription of these genes. Entertaining the possibility that differential transcription of genes may require some type of DNA conformation, K562 chromatin was used as a DNA template. While the results are still preliminary, we find that proper transcriptional control similar to that observed in vivo can now be demonstrated. These results were obtained by S1 nuclease mapping wherein we observed in vitro transcription of the fetal but not adult globin genes. We are currently exploring several approaches to increase the sensitivitiy of our transcription assay to insure that this important observation is not a result of our inability to detect adult globin transcription. These include utilization of a K562 nuclear extract for transcription, altering transcription conditions so chromatin is more accessible to transcription factors, and using high specific activity SP6 globin RNA probes to detect accurate transcription. The establishment of an in vitro transcription system which mimicks the K562 transcription pattern observed in vivo will enable us to examine molecules which surround a gene and its control region when that gene is in an active state and when it and other genes are in a repressed state.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 25052-02 LCB
formerly
Z01 AM 25052-01 LCB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Control of Membrane Transport by Osmotic Stress

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Joshua Zimmerberg	Guest Researcher	LCB, NIDDK
Others:	V.A. Parsegian	Chief, Section on Molecular Forces and Assembly	LCB, NIDDK

COOPERATING UNITS *(if any)*
Lab Theoretical Biology, NCI (Dr. A. Walter); Johns Hopkins University, Baltimore, MD (Dr. A. Harris); UCLA, CA (Dr. F. Bezanilla).

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NIDDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

To measure the internal volume change during opening and closing of ionic transmembrane channels, we have been subjecting perfused preparations to positive and negative osmotic stress. The extra work of channel opening under osmotic stress is measured as a shift in the current-voltage curve or as a bias in the open/closed statistics of a channel. Supression and enhancement of potassium channel conductance in the squid giant axon correspond nicely to the response one expects to osmotic stress. The data do not fit a simple blocking model. The channel volume infered has an upper bound of 1300 cubic angstroms. The mitochondrial voltage-dependent anion channel (VDAC) inserted into planar lipid bilayers shows a volume change of 20 to 40 thousand cubic angstroms. These are large changes inconsistent with traditional blocking or local gating models but supporting models with major closure of the channel space. A microcomputer data analysis system has been further adapted for these measurements.

The gap junction is the locus of direct transfer of ions and small molecules from cell to cell. We have (1) incorporated material from isolated gap junctions into vesicles, (2) applied a density shift technique to select vesicles containing large open channels, and (3) incorporated those channels into planar bilayers. A transport-specific purification of vesicles containing channels has been developed to improve the efficiency of the reconstitution. Predominantely three sizes of conductance changes were seen. They were 20-30, 60-70, and 110-130 pS, each having different degrees of voltage sensitivity, asymmmetry, and kinetics. Membranes containing several channels were anion selective.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 25053-02 LCB
formerly
Z01 AM 25053-01 LCB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Histamine Release from Beige Mouse Mast Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Joshua Zimmerberg	Guest Researcher	LCB, NIDDK
Other:	M. Curran	Guest Researcher	LCB, NIDDK

COOPERATING UNITS *(if any)*
Rush Medical College, Chicago, IL (Dr. F.S. Cohen); University of Texas, Galveston, TX (Dr. M. Brodwick).

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NIDDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are continuing to study the mast cell of the beige mouse, an animal model for the Chediak-Higashi syndrome in man. We study this cell because of its very large secretory granules, like the large granules of the neutrophils in the diseased state. One defect in that immuno-suppressed syndrome is failure in secretion. The large size of the beige mouse mast cell make possible detailed analysis of their exocytosis to the plasma membrane. Last year, we developed an instrumental array capable of simultaneous physiologic and anatomic real-time measurements of living cells with control of the internal millieu, using it to measure the capacitance of secretory and phagocytic peritoneal cells from the internally perfused mast cell from the beige mouse. Thus, we can combine biochemistry, anatomy, and physiology by performing perfusion studies on single active cells while recording both optical information (image, exocytosis, endocytosis, contraction), and electrical information (ionic currents, voltage clamp, capacitance) with 17 msec resolution. Preliminary studies reveal that the fusion of the secretory granule preceeds the swelling of that vesicle by 17-150 milliseconds. Granules which have been shrunken by hyperosmotic solutions also fuse to the cell membrane well before detectable swelling. Apparently in this system vesicle swelling is not needed for membrane fusion but may be required for the release of secretory products.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 25054-01 LCB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
G-protein Diffusion During Muscarinic Activation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Joshua Zimmerberg Guest Researcher LCB, NIDDK

COOPERATING UNITS *(if any)*
Laboratoire de Neurobiology, Ecole Normale Superieure, Paris, France (Dr. A. Marty).

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INSTITUTE AND LOCATION
NIDDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have studied the muscarinic response of rat lacrymal gland cells to acetylcholine with the tight-seal whole-cell technique. To measure the liberation of calcium from internal stores in that cell we monitored the calcium dependent K and C1 currents resulting from application of acetylcholine. Intracellular inclusion of IP2 liberated calcium. To show that polyphosphoinositide (PIP2) hydrolysis is a step in muscarinic activation we added neomycin, which binds to PIP2 and blocked activation by acetylcholine. The response to agonist diminished over several minutes after initiation of whole-cell dialysis. We found that the response was stable for some minutes, then decreased exponentially. The delay and time constant of the washout was directly proportional to the size of the pathway for diffusion and inversely proportional to the cell volume. It appeared that some soluble intracellular factor, needed after receptor activation, was needed for liberation of intracellular calcium. Attempts to constantly replenish PIP2 by inclusion of CTP, ATP, and inositol did not halt washout. Intracellular inclusion of ATP, GTP, cAMP, cGMP, together or alone, did not stop washout. We observed washout in one cell, gently removed the pipette after no further response to acetylcholine was seen, and sealed to the same cell a new pipette containing IP3. A rapid and sustained response was seen upon initiation of the whole cell mode. Intracellular inclusion of GTP-S potentiated the muscarinic response and slowed washout. We conclude that the washout is due to the loss of a diffusible factor which acts after muscarinic receptor activation and before polyphosphoinositol release. We suspect the action of the factor to involve a G protein.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 25055-01 LCB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Survey of Human Lymphoid Diseases for Human Pathogenic Retroviruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	David I. Cohen	Medical Staff Fellow	LCB, NIDDK
Others:	Jean-Pierre deVillartay	Guest Researcher	LCB, NIDDK

COOPERATING UNITS *(if any)*
BRMPDS, NCI (Dr. D. Longo), LMM, NIAID (Dr. M. Martin, Dr. H. Gendelman), ARB, NIDDK (Dr. G. Tsokos, Dr. F. Steinberg)

LAB/BRANCH
Laboratory of Chemical Biology

SECTION
Section on Molecular Biology and Genetics

INSTITUTE AND LOCATION
NIDDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Retroviruses have been postulated or established to play an etiologic role in many human lymphoid malignancies as well as in certain human autoimmune diseases. Despite this hypothesis, actual proof implicating human retroviruses in most of these diseases has remained elusive. Nevertheless, the recent isolation of two pathogenic retroviruses (HTLV I and II) from human T cell malignancies and the characterization of a third retrovirus (HIV) as the causative agent of acquired immunodeficiency disease (AIDS) has reawakened interest in this model. In particular, new data concerning the life cycle and growth of HIV have suggested new approaches to this problem. My laboratory has familiarized itself with these approaches by studying retroviral gene expression in several autoimmune mouse strains, and HIV. Now we are collecting human tissue with the goal of identifying new human pathogenic retroviruses.

To date, we have assayed DNA and RNA from patient with juvenile rheumatoid arthritis, rheumatoid arthritis, and systemic lupus for evidence of pathogenic retroviruses. We found that they do not contain sequences capable of cross-hybridizing with probes derived from HTLVI or HIV. In addition, the RNA did not contain one form of human endogenous retrovirus at elevated levels. While these studies probably rule out these, or closely related retroviruses, as causing these diseases, they do not invalidate the general model. Each new type of human pathogenic retrovirus has been distinctly different from previous isolates. The discovery of a pathogenic retrovirus in any of these diseases would be a dramatic breakthrough in understanding its etiology, and might establish a mechanism of preventing that disease through vaccination.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 25056-01 LCB

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Human T Cell Receptor Genes: Beyond Alpha and Beta

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	David I. Cohen	Medical Staff Fellow	LCB, NIDDK
Others:	Jean-Pierre deVillartay	Guest Researcher	LCB, NIDDK
	Ellen Nielsen	Biologist	LCB, NIDDK

COOPERATING UNITS *(if any)*

Metabolism Branch, NCI (Dr.S.Korsmeyer, Dr.T.Waldmann), LI, NIAID (Dr.R.Germain)

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Laboratory of Chemical Biology

SECTION

Section on Molecular Biology and Genetics

INSTITUTE AND LOCATION

NIDDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	1.6	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Important progress has been made in understanding the process by which T lymphocytes recognize antigen through the cloning and characterization of the alpha and beta genes of the T cell receptor for antigen (TCR). Despite this progress, the mechanism by which T lymphocytes recognize antigen in association with a product of the major histocompatibility locus (MHC), termed dual recognition, remains to be elucidated. This process has particular importance to our understanding allograft rejection and tolerance, which is critical for improving the success of renal and bone marrow transplants. It is becoming increasingly likely that understanding the expression of the alpha and beta genes alone will not explain dual recognition, and recent data from several laboratories suggest that additional TCR genes are involved. One such putative gene, termed gamma, has been isolated but remains poorly characterized. This project aims to define additional human T cell receptor genes with the goal of better understanding the process of dual recognition.

We have begun searching for additional human TCR genes which are postulated, like gamma, to participate in dual recognition. The initial approach has been to cross-hybridize a highly conserved region of mouse TCR genes selected by computer analysis with human genomic clones. This strategy, when applied previously by other laboratories for screening MHC genes, was useful in identifying new, unsuspected MHC genes. We have obtained a genomic clone, SLE1, which cross hybridizes with our murine TCR probe but is not the known human alpha, beta, or gamma genes. We are in the process of characterizing this clone to determine if it is a true TCR gene, but similar to authentic TCR genes, we have seen rearrangement of this gene in the DNA derived from some human T cell tumors. Subsequent studies will also employ antigen-specific human suppressor cells, which do not transcribe the beta TCR gene, and so must be using additional genes for antigen recognition.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 25057-01 LCB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Analysis of Self-Education

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

P.I.:	Jean-Pierre deVillartay	Guest Researcher	LCB, NIDDK
Others:	David I. Cohen	Medical Staff Fellow	LCB, NIDDK

COOPERATING UNITS *(if any)*
Scripps Institute, California (Dr. J. Sprent); Inserm U132, Paris, France (Dr. C. Griscelli), Cornell University Medical Center (Dr. M. Weksler).

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INSTITUTE AND LOCATION
NIDDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The process of self-education selects appropriate antigen-specific T cell receptors (TCRs) which are tolerant to determinants of the major histocompatibility locus (MHC) alone, but which can interact with self-MHC in the presence of antigen.

Recently the TCR has been characterized at the molecular level. The TCR is constituted by the association of at least two polypeptide chains, alpha and beta, which contribute to the specificity of the T cell. These two polypeptides are encoded by two distinct sets of genes. In addition to these two subunits, a third set of genes encoding a putative TCR molecule, called gamma chain, has been found expressed at the mRNA level in certain populations of T cells. Nevertheless, the protein product of this gene as well as its function are not known to date. The genes encoding these three polypeptides are present in an unrearranged form in germline DNA and undergo specific rearrangement in DNA of cells of the T cell lineage.

The pattern of these rearrangements presumably controls the specificity and tolerance of the T cells. We have described a situation in humans following MHC haploidentical Bone Marrow Transplantation (BMT) in which tolerance to donor MHC determinants did not occur, leading to the circulation of donor T cells displaying reactivity towards donor histocompatibility determinants. An autoreactive T cell line from this patient has been grown in vitro. We propose to study the expression of the TCR genes in this autoreactive cell line. The same study will also be done in mice in which it has been possible to induce severe auto-Graft-versus-Host (GVH) reactions in vivo after syngeneic BMT. The study of TCR expression in these two abnormal situations should give us some insight into the process of normal self-education at the molecular level, and should contribute to transplantation therapy in general.

PHS 6040 (Rev 1/84)

GPO 904 917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 25058-01 LCB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Laboratory and Clinical Models for the Study of Globin Gene Expression

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Griffin P. Rodgers	Guest Researcher Robert Wood Johnson Fellow	LCB, NIDDK
Others:	Constance T. Noguchi	Research Physicist	LCB, NIDDK
	Nadera Ahmed	Guest Researcher	LCB, NIDDK
	Alan N. Schechter	Chief	LCB, NIDDK

COOPERATING UNITS *(if any)*
Lab. Mol. Genetics, NICHD (Dr. H. Westphal); MRC Unit, Univ. of West Indies, Kingston, Jamaica (Dr. G. Serjeant).

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Laboratory of Chemical Biology

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INSTITUTE AND LOCATION
NIDDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	0.7	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are investigating the molecular mechanisms which control the individual and total concentrations of hemoglobins in human erythrocytes. In addition, we are studying the effects of functional alpha globin gene number, fetal hemoglobin (HbF) levels and the extent of red cell heterogeneity on the various manifestations of sickle cell disease and its genetic variants. The levels of each of the normal hemoglobins (A, A2, F) are determined by controls at the level of transcription and/or translation of the globin genes, as well as by factors that regulate protein degradation. The study of the control of hemoglobin levels has direct relevance to various hemoglobinopathies especially thalassemia and sickle cell disease. In addition, these studies are of potential relevance to the more general question of control of gene expression in eukaryotic cells. For our experimental system, we are using the K562 human leukemic cell line, as well as peripheral blood from individuals with sickle cell disease. We are studying the effects of short-term and long-term exposure of these cells to 5-azacytidine on their phenotype and the factors that control globin gene transcription. Concurrently, we are also attempting to develop a sickle cell mouse model by the introduction of a cloned human sickle globin gene into the mouse germ line by the microinjection of DNA into the pronuclei of fertilized eggs. The establishment of such a model would allow for basic and fundamental questions to be asked about the molecular, cellular and physiologic aspects of the disease, as well as provide an in vivo system to monitor the effects of potential therapy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 25059-01 LCB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Oncogenes and the Control of Globin Gene Expression

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	Harish Dave	Visiting Fellow	LCB, NIDDK
Others:	Alan N. Schechter	Chief	LCB, NIDDK

COOPERATING UNITS *(if any)*

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Laboratory of Chemical Biology

SECTION
Section on Molecular Biology and Genetics

INSTITUTE AND LOCATION
NIDDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Humans undergo two developmental switches in their hemoglobin phenotype, the embryonic to fetal switch occuring in early gestation and the fetal to adult switch occuring around the time of birth. Gene regulation has two main components, namely cis-acting DNA sequences and trans-acting molecules (presumably proteins that interact with DNA sequences in a specific manner to control gene expression. The K562 human leukemia cell line expresses all globin genes other than the adult beta-globin. Previous work from this laboratory showed that the beta-globin gene of K562 cells functions normally in a heterologous expression system. Elucidation of the mechanism of failure of beta-globin gene expression in K562 cells may provide an insight into globin gene expression and switching in normal erythroid cells.

The human adenovirus E1a protein product acts in trans on the transcription of other viral genes as well as certain resident cellular genes such as hsp 70. An enhancer deficient human beta-globin gene is not transcribed in HeLa cells; however, cotransfection with the E1a gene results in easily detectable beta-globin mRNA. The c-myc protein nuclear matrix product has limited homology to adenovirus E1a protein and like E1a can induce expression from the hsp 70 promoter and can complement c-ras in transforming primary cells. We propose to transfect a hybrid myc plasmid with a dexamethasone responsive regulatory element (MMTV-Xba-myc) into K562 cells and study epsilon and beta-globin gene expression upon steroid induction. Hybrid epsilon- and beta-CAT plasmids will be co-transfected with MMTV-Xba-myc and the level of CAT activity assayed in heterologous systems. No suitable human erythroid cell lines expressing only beta-globin are available. Such line(s) will be established using MMTV-Xba-myc and a c-ras containing oncogene. The steroid inducible MMTV element should permit mimicry of the in vivo decrease in c-myc mRNA level seen with differentiation, as well as supply an adult hemoglobin forming cell line.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 25060-01 LCB

PERIOD COVERED: October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Transcriptional Control of Globin Genes in In Vitro Assays From K562 Cell Extracts

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Yuko Wada	Visiting Fellow	LCB, NIDDK
Others:	Helena Mishoe	Senior Staff Fellow	LCB, NIDDK
	Barbara Torain	Biological Lab Technician	LCB, NIDDK
	Constance T. Noguchi	Research Physicist	LCB, NIDDK

COOPERATING UNITS *(if any)*:

LAB/BRANCH: Laboratory of Chemical Biology

SECTION: Section on Molecular Biology and Genetics

INSTITUTE AND LOCATION: NIDDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.2	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

It has been demonstrated that K562 cells express epsilon- and gamma-globin genes but do not express beta-globin genes. The regulation of gene expression occurs at the level of transcription. We have set up a cell-free in vitro transcription system from K562 cells to determine the requirements for globin mRNA synthesis. As an initial attempt, we have prepared extracts from nuclei of both hemin-induced and uninduced K562 cells. The nuclear extracts could direct accurate transcription initiation in vitro from the epsilon-globin gene promoter without supplement with whole cell extracts. The results up to the present point to the existence of a globin gene expression regulatory factor(s) in K562 nuclear extracts. The in vitro transcription system will be used as an assay system for the isolation and characterization of such factors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 25061-01 LCB

PERIOD COVERED: October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Isolation of Embryonic Globin Transcriptional Factors by Subtractive cDNA Cloning

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Yongji Wu	Visiting Fellow	LCB, NIDDK
Others:	Yuko Wada	Visiting Fellow	LCB, NIDDK
	Barbara Torain	Biological Lab Technician	LCB, NIDDK
	Constance T. Noguchi	Research Physicist	LCB, NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH: Laboratory of Chemical Biology

SECTION: Section on Molecular Biology and Genetics

INSTITUTE AND LOCATION: NIDDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.2	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The transcription of human globin genes may involve the complex interaction of a variety of factors. The K562 human erythroleukemia cell line can serve as a model for the study of globin gene expression. The K562 cell line can be induced by hemin to accumulate embryonic and fetal hemoglobin, but not adult hemoglobin. It has been demonstrated that the beta-globin gene is intact but inactive in these cells. The cloned K562 beta-globin gene is expressed in COS cells and the transcriptionally inactive beta-globin gene in HEL cells is activated in MEL x HEL hybrids suggesting that the induction of beta-globin gene expression perhaps requires a specific transcriptional factor. The zeta-globin gene promoter functions after microinjection into oocytes but not after transcription into HeLa or COS cells also suggesting that there might be transcriptional factors specific for embryonic genes.

The current study assumes that induced K562 cells contain transcriptional factors specific for embryonic globin genes, which are absent or present only at low levels in uninduced K562 cells. We are preparing cDNA from the mRNA of induced K562 cells. The mRNA from uninduced K562 cells will be used to subtract the background corresponding to proteins present in both the induced and uninduced K562 cells. The remaining cDNA will be cloned into a vector (lambda gt10) to replicate enough copies for screening with mRNA or cDNA, from both the induced and uninduced K562 cells. Those cDNA clones which are differentially expressed will be further characterized by transfecting back into K562 cells or other hemoglobin or non-hemoglobin producing cell lines, or by inserting into a protein expression vector (lambda gt11) so that the protein product can be examined.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 25062-01 LCB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Factors Affecting Mouse Beta-Globin Gene Expression

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Patricia Berg-Lovett	Senior Staff Fellow	LCB, NIDDK
Others:	Alan N. Schechter	Chief	LCB, NIDDK

COOPERATING UNITS *(if any)*
Laboratory of Molecular Hematology, NHLBI (Drs. D. Williams, Dr. D. Kuebbing and W.F. Anderson).

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Section on Molecular Biology and Genetics

INSTITUTE AND LOCATION
NIDDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In order to analyze the sequence requirements for induction of the mouse beta(maj)-globin gene, we have developed a transient assay system in mouse erythroleukemia (MEL) cells. These cells, which have been transformed by the Friend virus complex, can be chemically induced to undergo terminal differentiation during which transcription of endogenous alpha and beta globin genes is greatly increased. If we can mimic this effect in a transient assay, which only requires 4 to 5 days, then it should be possible to quickly and accurately analyze plasmid constructions with varying amounts of DNA 5' or 3' to the beta-globin promoter to determine what regions are required for induction. Transient assay conditions have been optimized for both uninduced and induced MEL cells and we currently wish to determine if induction affects genes located on transfected plasmids which in these experiments remain episomal.

We have previously shown that DNA sequences known as enhancers increase the activity of the mouse beta-globin promoter in transient assays. Enhancers are cis-acting DNA sequences which act at the level of transcription to increase gene expression. They can function in either orientation both 3' and 5' to the target gene and their level of activation is relatively independent of position. While there is no high degree of sequence homology among the presently identified enhancers, two categories of short "core" regions have been observed. We are interested in determining if any other common features of enhancer DNA sequences exist and, if so, whether they might suggest possible mechanisms of enhancer activation of the mouse beta-globin promoter as well as other enhancer activated promoters. Analysis of five enhancers has shown that each exhibits dyad symmetry; we are extending this analysis to include additional enhancers. We have also shown that known enhancer mutants exhibit less dyad symmetry than the wild type enhancer, suggesting there may be a correlation between enhancer function and degree of dyad symmetry.

ANNUAL REPORT OF THE LABORATORY OF CHEMICAL PHYSICS
NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

Research in the Laboratory of Chemical Physics is directed toward the application of physical methods, principally spectroscopic, to the study of the structural and dynamical properties of both molecular and cellular systems. Theoretical and experimental research is carried out. A major objective of the former is to enhance the ability to uniquely interpret spectroscopic and light scattering data. Experimental spectroscopic techniques include nuclear and electron magnetic resonance and infrared, Raman and ultraviolet spectroscopy; specialized techniques involve nanosecond absorption spectroscopy, electric-field-induced linear dichroism, x-ray spectroscopy, and multiphoton ionization spectroscopy. Systems currently under investigation include model and intact cell membrane assemblies, proteins, nucleic acids, red blood cells, retinal photoreceptors, and various small, prototypical biological molecules. This summary of the Laboratory's work starts with new theoretical developments by Szabo and his associates and continues with the report of the experimental validation of earlier theoretical achievements of Charney and his colleagues on the behavior of polyelectrolytes in electric fields. Various semi-theoretical and computational accomplishments by other members of the scientific staff are interwoven with their experimental studies.

Studies of the structure and internal motion in lipid bilayers are current in two projects. The theoretical studies by Szabo and Pastor (FDA) are demonstrating the need for computer simulations to model the fast motional dynamics of acyl chains in the bilayers to resolve the ambiguities that arise from experimental relaxation time observations. Ordinary computer simulations of macromolecular motion does not, unfortunately, permit the calculation of their free energy because the entropy cannot be expressed as an equilibrium average. Szabo and collaborators at Rutger University are engaged in a project to determine the entropy using moments of the internal coordinate displacements obtained in the dynamics simulation. Preliminary applications to model systems are promising. Lamm and Szabo have also developed a novel stochastic dynamic approach to the evaluation of thermodynamic properties of systems where quantum effects are significant.

Szabo has also been engaged in a theoretical study of quite a different sort. Together with a group at Harvard University he has successfully developed a quantitative model that accounts for the variation of the observed association rates of α and β globin subunits in hemoglobin that account for its variation with surface charge in mutants with different surface charges and explains why the dissociation rate is charge independent.

Two experimental studies by Charney and his coworkers have demonstrated the validity of the theoretical treatment of the high electric field polarization of nucleic acids [Rau, D. C. and Charney, E. Macromolecules 16:1653 (1983)]. In the first, it was shown that extrapolation of electric field data obtained in electric dichroism

measurements at low fields on double helical poly (dG-dC) in the B conformation in a way consistent with the theory yields structural parameters entirely consistent with x-ray structures obtained from nucleoside oligomer crystals of dG-dC in B form. In the second, solution electric dichroism measurements on DNA in the A form gave the same consistency with nucleoside crystals of DNA oligomers in the A form. Two other complimentary theoretical and experimental studies are underway. One in collaboration with Professor George Pack, a guest researcher from the University of Illinois, involves computing the charge distribution around a highly charged polymer like DNA and its perturbation by an electric field during a dichroism experiment. The other study, in collaboration with Sybren Wijmenga of Leiden, Holland, involves an experimental examination of the effect of polymer charge density on electric field orientation using hyaluronic acid as the experimental probe.

These new methods have been developed by Bax and his colleagues (Lerner, Subramanian and Summers) that permits the regular ^{1}H proton NMR spectrum of a molecule to be separated in a number of subspectra of limited fragments of the molecule. This simplifies spectral assignment and permits the exact measurement of coupling constants for spectral regions that were previously not accessible. The method lengthens the apparent relaxation time of the nuclei during the mixing process which yields a large sensitivity enhancement in the study of macromolecules. Lerner has used the combination of all three new methods to completely assign ^{1}H and ^{13}C NMR spectra of a number of complex oligosaccharides.

New two-dimensional NMR methods utilizing protons for indirect detection of nuclei with a low magnetogyric ration (e.g. ^{13}C, ^{15}N) have been developed by Bax and Subramanian. These methods offer an increase in sensitivity of more than an order of magnitude relative to other modern NMR techniques. The methods can be used for detecting either direct (one-bond) connectivity or long range (multiple-bond) connectivity. This latter application has been shown to be extremely useful for structure determination of unknown compounds. For example, Lerner and Bax have used this technique to measure J couplings in oligosaccharides and to compare the conformation of the sugar units with these of the corresponding monomeric sugars.

Using the method of spin labeling for electron paramagnetic resonance (EPR) measurements, Kon and his associates have looked for evidence of a proposed mechanism of copper induced hemolysis in the hemolytic anemia complication that arises in Wilson's disease and other forms of copper intoxication. Measurements showed a dose (of Cu(II)) dependent cell deformability as well as echinocyte formation. Using various reagents, it was shown that a part of the effect of Cu(II) is the result of formation of Cu(II) coordination complex in the membrane, which can be reversed by chelating agents, while others are due to disulfide bond formation and/or CU(II) complexation involving sulfhydryl groups, which can not be reversed by chelating agents but are reversible by a reducing agent. In another study using the same technique, Kon has found that the membrane fluidity of human peripheral blood leukocytes does not appear to correlate with properties of monocyte differentiation to macrophage in vitro, as has generally been surmised.

Eaton and Hofrichter and their colleagues have continued their productive series of research investigations into the chemistry and biology of hemoglobin, especially as it pertains to sickling of red blood cells in sickle-cell anemia. Using state-of-art physical methods and computational facilities, the major aim of the work is to elucidate the molecular mechanism of hemoglobin S polymerization in order to provide a rationale for the development of agents that can be used in the treatment of patients with sickle cell disease.

Experiments on solutions of purified hemoglobin S show that the solubility at partial saturation with carbon monoxide created by photolysis with a laser beam is the same as that for hemoglobin S partially saturated with oxygen by reducing the oxygen pressure. Thus, photo-desaturation of the carbon monoxide complex closely simulates deoxygenation. Measurements on cells show the enormous hysteresis for the formation and disappearance of polymer in desaturation and resaturation experiments. The saturation at which polymer forms is always lower than the saturation at which polymer disappears, as expected from kinetic studies, since there is a delay prior to polymer formation but no delay associated with depolymerization. One of the major findings is that cellular deformation and reformation is found to be simultaneous with the appearance and disappearance of polymer, so that cell deformation may be considered a reliable indicator of intracellular gelation.

One of the most interesting set of experiments were those done on cells containing hemoglobin S in which polymer formation was monitored as a function of oxygen saturation which indicate that gelation does not occur in most cells in vivo because the delay times are sufficiently long for them to return to the lungs and become reoxygenated before any polymer has formed.

Henry's and Eaton's molecular dynamics project is also directed at the study of blood hemoglobin. The technique is being used to simulate internal motions in macromolecules by explicitly integrating Newton's equations of motion for all the atoms under the influence of interatomic forces described by empirical potential functions. One of the newest results comes from a study of the heme conformational change in a hemoglobin alpha subunit following photodissociation of bound carbon monoxide ligands. It was found that the motion of the iron atom out of the heme plane takes place on a sub-picosecond time scale. This project is currently being extended to simulations of the complete hemoglobin tetramer.

Henry, in collaboration with Robin Hochstrasser (University of Pennsylvania), has performed molecular dynamics simulations of atomic motions in sperm whale myoglobin in order to study the influence of internal motions on the observed decays of fluorescence intensity and anisotropy of tryptophans in this protein. The dynamics simulations predict the existence of relatively slow fluctuations in the instantaneous energy transfer rate with a consequent non-exponentiality in the early fluorescence intensity decays.

In another collaboration with Hochstrasser, Henry and Eaton have used the molecular dynamics technique to determine the lifetime of the

thermal excitation of a heme which is coupled to the surrounding protein. Simulations have been performed on both myoglobin and cytochrome-c in which the energy equivalent of single photon of 530 nm or 353 nm light (54 and 81 kcal/mol, respectively) was instantaneously injected into the heme group. The results indicated that temperature rises of many hundreds of degrees K may persist in laser-excited hemes (and by extension other chromophores bound to protein matrices) for tens of picoseconds. Many of the spectral transients seen in picosecond and sub-picosecond laser photolysis experiments may therefore be attributed to thermal effects in the excited chromophore rather than conformational changes in the surrounding protein.

One of the principal objectives of the molecular dynamics studies reported above is to help interpret experimental observations in the fast transient spectra that Hofrichter, Mozzarelli, Eaton and others have been doing on laser photodissociated hemoglobin-ligand complexes. Among these studies are those of the temperature-dependence of the geminate rebinding of CO to myoglobin and hemoglobin. New results have been obtained that are consistent with a model in which relaxation of the protein tertiary structure reduces the rate at which ligands bind to the heme, but not with a multiple well model.

Hofrichter has developed a light scattering technique capable of monitoring polymerization reactions in volumes as small as 10^{-10}cc and has used it to study the assembly kinetics of hemoglobin S when either the initial or the final state is partially liganded. This experiment takes advantage of the sensitivity of the polymerization kinetics to small amounts of polymer to detect the "threshold" saturation at which polymers first are stable thermodynamically in partially saturated sickle cells. Initial results show clearly that cells deform ("sickle") when even small amounts of polymer form inside them, and they do not unsickle until all the polymer is dissolved.

Somewhat less rapid than photo-induced changes in hemoglobin, but nevertheless rapid photoreactions have been the subject of investigations in still an entirely different system by Hagins, Yoshikami and Foster. A new method for measuring just how much free cGMP is hydrolyzed during the light responses of vertebrate retinal rods has been developed. The heat released during a light response is measured directly by a new pyroelectric sensor whose sensitivity is at least three orders of magnitude greater than conventional heat sensors and has such a fast response that it can distinguish heat generated in the rod outer segments from that produced by other layers of the retina. Over a wide range of stimulus intensities, the total cGMP hydrolysis is less than 1 micromolar in rod cytoplasm.

The cytoplasmic Ca^{++} level in retinal rods is at least partly regulated by a Na:Ca exchange pump in the plasma membrane of the outer segments. By using x-ray microanalysis to determine the driving force for Na:Ca exchange, it is possible to set an upper limit on the cytoplasmic Ca^{++} level. It has been possible to show that Na:Ca exchange will tend to hold the cytoplasmic Ca^{++} level at about 5-10 micromolar in the dark over a wide range of external calcium concentrations. Agents tending to raise the cytoplasmic level of cGMP tend to raise the intracellular

$[Na^+]$ and to lower the Cl^- potential, much like low external Ca^{++}.

The most optically significant organic compound in the retina of higher arrivals including humans is retinene, a conjugated polyene. Polymers are also formed in steroidal and photosynthetic systems. McDiarmid and her coworkers have concentrated for a number of years in studying the photophysics of the precursor polyenes. This year with Sabljic the Rydberg spectra of cis-hexatriene obtained with one photon optical and two and three photon resonant multiphoton ionization spectroscopies excited using linearly and circularly polarized light were obtained and analyzed. From comparisons of these results two 3d- and one 4s- Rydberg transitions were found to be active. This, in turn, permitted definitive resolution of the 3d-4s uncertainty remaining in the analyses of trans-hexatriene spectra. In conjunction with the investigations of the electronic excited states of the hexatrienes, it became necessary to remeasure and reassign the vibrational (infrared and Raman) spectra of these molecules. The new assignments are internally consistent. With these results it becomes possible to evaluate some proposed theoretical models for predicting vibrational frequencies. Correlations of all known data concerning the lower Rydberg states of the family of small conjugated polyenes have now been completed. From this it became possible to deduce that in these planar molecules, increasing the conjugation length perturbs the energy levels of the in-plane Rydberg orbitals but not those of the out-of-plane orbitals. Merely enlarging the molecule by methyl substitution appears not to alter either set of energy levels. The results are important in the study of theoretical models of excited electronic states of isolated molecules and can probably be applied to the understanding of the changes in electronic properties of isolated molecules on attachment to surfaces or condensation into solids.

Returning once again to spectroscopic studies in the low frequency region of the electromagnetic spectrum, we note that Levin and his coworkers have concentrated on vibrational Raman and infrared investigations of the dynamical, conformational and packing properties associated with lipid-lipid interactions in membrane assemblies. Levin and Harris have examined the thermotropic behavior of a series of n-alkyphosphocholines dispersed in excess water by vibrational Raman spectroscopy. The single chain, interdigitated system, which include the odd-numbered n-C(15), n-C(17), and n-C(19) phosphocholine homologs, exhibit sharp lamellar to micellar transition forms. Raman spectra provided clear and definitive spectral patterns enabling the subcell chain packing characteristics of each polymorph to be characterized. A related study of aqueous dispersions of methyl derivatives of di-O-hexadecylglycero-phosphocholine, an ether lipid in which a methyl group is substituted at the 1,2 or 3 position of the glycerol backbone was carried out by Levin and Lewis.

Levin, with O'Leary and Ross, investigated the effects of cis- and trans-9, 10 tetradecenols on the phase transitions of dimyristoyl-, dipalmitoyl- and distearoylphosphatidylcholines using high sensitivity scanning calorimetry and Raman spectroscopy. These results are interpreted in terms of the structural changes which are introduced by the alcohols into the gel (L_{β},), and ripple (P_{β}) phases, and the consequent effects of these structural changes on the pretransition and

the gel to liquid crystalline phase transition. The data clearly demonstrate that caution is necessary in applying information on lipid-anesthetic interactions obtained from model membranes to the problem of clinical anesthesia, since qualitatively different results may be obtained when lipids of differing acyl chain lengths are employed.

Adams and Levin have investigated the problem of the analysis of intensity data from Raman spectra of model phospholipid membranes. Data from integrated vibrational intensity studies (temperature programmed Raman spectroscopic procedures) indicate that there are much more complicated changes occurring in model phospholipid membranes than has been shown by the conventional analysis of peak ratio and frequency shifts. It appears that the subtransition (the lowest temperature change shown), at least in the case of dimyristoyl (C14)- and dipalmitoyl (C16)-phosphatidylcholine, is a change in crystalline form from orthorhombic to monoclinic subcell in preparation for the shift to a quaishexagonal form (the gel state). The data also support the concept that dehydration effects determine the occurrence of a subtransition.

Ziffer and his collaborators have continued to examine the enantioselective hydrolysis of esters. These hydrolyses are catalyzed by enzymes in the mold Rhizopus nigricans. A quantitative analysis was carried out to determine the relative contributions that electronic, steric and polarizability factors make both to the formation of the enzyme-substrate complex and to the relative rates of hydrolysis of the enantiomers. The data show the relative importance of steric, electronic and polarizability factors and demonstrate that it is possible to predict the optical purity of an alcohol formed using standard value of the size, electronegativity and polarizability of substituents. To complement the methodolgy developed for the preparation of chiral allylic alcohols, a preliminary study was carried out to demonstrate that the ketal Claisen rearrangement (a rearrangement of vinyl ethers of allylic alcohols), a reaction that had not previously received much attention, can be used for the synthesis of sesquiterpenes such as cuparene, laurene, bazzanene and trichodiene. Studies have also been started to investigate synthetic procedures that enable one to transfer the chirality of the hydroxyl group to newly formed carbon-carbon bonds.

Scientist Emeritus, Weiss, in cooperation with Professor Cook at the University of Wisconsin is continuing exploration of the reaction of dimethyl 3-ketoglutarate with 1,2-dicarbonyl compounds with the goal being the exploitation of this remarkably facile reaction for the stereospecific preparation of additional ring systems composed of five-membered rings. This reaction has established itself as a convenient, versatile and reliable method for the stereospecific synthesis of symmetrically constituted diketones derived from the ring-system cis-bicyclo[3.3.0]-octane, which consists of two five-membered rings sharing two adjacent carbon atoms; it is often called the Weiss reaction. Further elaboration yields many other substances containing ring systems made up from cyclopentane units. This class of so-called cyclopentanoids includes many biomedically significant compounds; see, e.g., the prostaglandins, prostacyclin and its synthetic analogs, the tumor-inhibiting mold metabolite quadrone, etc. Contributions to the methodology of their synthesis are thus potentially important. Progress has been made in the

further utilization of this reaction for the synthesis of otherwise poorly accessible substances. Techniques for the introduction of double bonds into predetermined sites of the molecules are now well in hand.

Quantum molecular computations of stable configurations of biologically relevant molecules by Sharpless and his collaborators continues to provide insight into the relation between molecular structure and activity. New computations on the factors important to aldose reductase inhibitors indicate that important structural features which may enhance activity include the presence of a spiro carbon atom, as well as the presence of a pyrimidine unit, and, in addition, halogenation. Strain energies and quantum calculations on colchicine, which binds to tubulin, and isocolchicine, which does not, have been essentially completed. The central torsion angle at the minimum of energy is ca. 23 degrees in conchicine and about 30 degrees in isocholchicine. The isocolchicine is more strained by ca.8 kcals./mol. The factor which differentiates the binding ability of the two isomers seems to be different inter-oxygen distances, which affect hydrogen binding ability in isocolchicine. Strain energy and quantum calculations are being carried out on some of the new compounds now being feverishly sought in the fight against AIDS.

In addition to the major computational facilities of the NIH Division of Computer Research and Training, the scientific staff of this laboratory and of the Laboratory of Molecular Biology are heavily dependent on the PDP 11/70 minicomputer facilities housed in our building as well as a myriad of terminals and auxiliary computing facilities. Jennings with the help and cooperation of other members of the scientific staff, and of the DCRT staff, is responsible for the continued useful operation and updating of these facilities. An active study of a replacement system for the PDP 11/70 is now underway as maintenance of the PDP 11/70 becomes increasingly problematic and the facility becomes increasingly insufficient for the needs of the two laboratories.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-DK-29001-14-LCP
Formerly
Z01-AM-29001-13-LCP

PERIOD COVERED
October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular dynamics and vibrational characteristics of membrane assemblies

PRINCIPAL INVESTIGATOR *(List other professional personnal below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI :	Ira W. Levin	Research Chemist	LCP-NIDDK
OTHERS:	Neil E. Lewis	Visiting Fellow	LCP-NIDDK
	Peter M. Green	Staff Fellow	LCP-NIDDK

COOPERATING UNITS *(if any)* R. Adams, LCP-NIDDK; Clifford J. Steer, LBM-NIDDK; C. Huang, School of Medicine, Univ. of VA; G. DeHaas, Univ. of Utrecht; William C. Harris, Natl. Science Foundation; James S. Vincent, Univ. of MD; W. H. Kirchhoff, National Bureau of Standards; P. D. Ross, LMB-NIDDK; T. J. O'Leary, FDA

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Laboratory of Chemical Physics

SECTION
Section on Molecular Biophysics

INSTITUTE AND LOCATION
NIH, NIDDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	3	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Vibrational Raman and infrared spectroscopy are used to probe the dynamical, conformational and packing properties associated with lipid-lipid interactions in membrane assemblies. For example, the thermotropic behavior of a series of n-alkylphosphocholines dispersed in excess water has been examined by vibrational Raman spectroscopy in both the C-H stretching (2800-3100 cm^{-1}) and the C-C stretching (1000-1200 cm^{-1}) mode regions, spectral intervals characteristic of alkyl chain lateral interactions and intrachain disorder, respectively. The single chain, interdigitated systems, which include the odd-numbered n-C(15), n-C(17), and n-C(19) phosphocholine homologs, exhibit sharp lamellar to micellar transitions at 13.8, 26.1 and 33.2°C, respectively. Additional polymorphic forms, whose transition temperatures are 4 to 15°C below the more stable structures, were identified. Raman spectra provided clear and definitive spectral patterns enabling the subcell chain packing characteristics of each polymorph to be characterized. For n-C(15) phosphocholine the higher melting interdigitated lamellae exhibit spectra characteristic of a hybrid lattice composed of both orthorhombic (or monoclinic) and hexagonally packed domains. The less stable polymorphic form assumes, however, a lattice whose alkyl chains pack in triclinic subcells. The alkyl chains of the less stable polymorphic forms of the n-C(17) and n-C(19) phosphocholines are hexagonally packed, while the higher melting, more stable lamellae exhibit spectra suggestive of the hybrid lattice structure observed for the n-C(15) system. Spectral splittings in the ~3040 cm^{-1} choline methyl asymmetric stretching mode region indicate an ordered, perhaps dehydrated, headgroup for the polymorphs in the hybrid, higher melting lamellae. In contrast to the n-C(17) and n-C(19) phosphocholine lamellae, the low temperature triclinic polymorph of the n-C(15) system displays a variable alkyl chain reorganization at 6°C.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-DK-29002-13-LCP
Formerly
Z01-AM-29002-12-LCP

PERIOD COVERED
October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Chemistry of natural compounds, and synthetic organic chemistry

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: Ulrich Weiss Research Chemist (Scientist Emeritus) LCP-NIDDK

COOPERATING UNITS *(if any)* James M. Cook, Dept. of Chem., University of Wisconsin-Milwaukee
K.M. Madyastha, Dept. of Organic Chem., Indian Institute of Science, Bangalore
Surendra P. Bhatnagar and B.B. Singh, Reckitt & Colman of India, Bangalore
H.M. Fales, Laboratory of Chemistry, NHLBI

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INSTITUTE AND LOCATION
NIH, NIDDK, Bethesda, Maryland 20892

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1	1	

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In continued cooperation with Dr. Cook and his group, the synthesis of di- and polycyclic ring systems composed of cyclopentane rings has been developed further, and work on several novel systems of this type has been initiated.

Cooperation with colleagues in Bangalore, India, in the field of alkaloid chemistry and biosynthesis is to be resumed after a temporary interruption.

In cooperation with Professor Merlini, the study of photosensitizing perylene quinones from molds has been continued. A comprehensive review of this class of natural compounds is being prepared; no such review exist so far.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-DK-29005-12-LCP
Formerly
Z01-AM-29005-11-LCP

PERIOD COVERED
October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Asymmetric synthesis; structure, stereochemistry and NMR

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI : Herman Ziffer Research Chemist LCP-NIDDK

COOPERATING UNITS *(if any)*
Prof. Marvin Charton, Chemistry Department, Pratt Inst., Brooklyn, N.Y.
Prof. Paul F. Schuda, Chemistry Department, Univ. of Md., College Park, MD.

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SECTION
Section on Molecular Biophysics

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NIH, NIDDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In developing methodologies to prepare chiral alcohols and to make tentative assignments of their absolute stereochemistry (based on their method of preparation), we have continued to examine the enantioselective hydrolysis of esters. These hydrolyses are catalyzed by enzymes in the mold Rhizopus nigricans, and the configuration of the alcohol formed can be predicted from considerations of the effective sizes of substituents on the carbinol carbon. While data on the absolute stereochemistry of the more rapidly hydrolyzed enantiomer of the ester were consistent with the rule, a quantitative analyis was carried out to determine the relative contributions that electronic, steric and polarizability factors make both to the formation of the enzyme-substrate complex and to the relative rates of hydrolysis of the enantiomers. Data on the optical purities of the alcohols formed and the percent hydrolysis were employed to calculate an "E" value which describes the relative rates of hydrolysis of the two acetate enantiomers. The data show the relative importance of steric, electronic and polarizability factors and demonstrate that it is possible to predict the optical purity of an alcohol formed using standard values of the size, electronegativity and polarizability of substituents. To complement the methodolgy developed for the preparation of chiral allylic alcohols described, we carried out a preliminary study to demonstrate that the ketal Claisen rearrangement (a rearrangement of vinyl ethers of allylic alcohols), a reaction that had not previously received much attention, can be used for the synthesis of several sesquiterpenes, cuparene, laurene, bazzanene and trichodiene. Each of these natural products contain a quaternary carbon atom. We propose to employ the established chirality of an allylic alcohol to control the absolute stereochemistry of a quaternary carbon atom formed in the rearrangement.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-DK-29006-16-LCP
Formerly
Z01-AM-29006-15-LCP

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The structure and dynamics properties of macromolecules

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. :	Elliot Charney	Research Chemist	LCP-NIDDK
Others:	Sybren Wijmenga	Visiting Fellow	LCP-NIDDK
	George Pack	Guest Researcher	University of IL

COOPERATING UNITS *(if any)*
H-H. Chen, George Mason University, Fairfax, VA
E. Henry, LCP-NIDDK
D. C. Rau, LCB-NIDDK

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SECTION
Spectroscopy and Structure

INSTITUTE AND LOCATION
NIH, NIDDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	2.5	1.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A. Macromolecular structure, dynamics and polyelectrolyte properties of large biological polymers, in particular, polynucleotides and nucleic acids are being studied by electric-field induced dichroism and birefringence methods. Theoretical and computational methods supplement the experimental work.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-DK-29007-15-LCP
Formerly
Z01-AM-29007-14-LCP

PERIOD COVERED
October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structure and interaction of biomolecules

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Hideo Kon	Research Chemist	LCP-NIDDK
OTHERS:	Toshiharu Ito	Visiting Fellow	LCP-NIDDK

COOPERATING UNITS *(if any)*

Tsuneo Takahashi, American Red Cross

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SECTION
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INSTITUTE AND LOCATION
NIH, NIDDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	2	0

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have applied the spin label EPR method to study the effect of Cu(II) ion on the red cell deformability loss and morphological changes to clarify the mode of Cu(II) interaction with red blood cells under pre-lytic conditions.

The spin label method was also applied to the study of a correlation between the membrane fluidity and the cell differentiation in monocytes.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-DK-29008-15-LCP
Formerly
Z01-AM-29008-14 LCP

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Electronic and molecular structural investigations

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. : Ruth McDiarmid Research Chemist LCP-NIDDK

OTHERS: Aleksandar Sabljic Visiting Fellow LCP-NIDDK

COOPERATING UNITS *(if any)*

Leo Klasinc - Rudjer Boskovic Institute, Yugoslavia

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Laboratory of Chemical Physics

SECTION
Spectroscopy and Structure

INSTITUTE AND LOCATION
NIH, NIDDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.25	1.25	

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

1. The infrared and Raman spectra of cis- and trans-hexatriene were remeasured and reassigned. Past assignment(s) were shown to be slightly, but significantly, in error.

2. The lower Rydberg transitions of cis-hexatriene were studied in detail. Firm assignments were obtained for the $3p(A_1)$ and $3d(A_2)$ Rydberg states. The 3s, $3p'$, $3d'$ and 4s states were also observed.

3. A synthesis of the effect of different types of substitutional perturbations on the lower Rydberg spectra of conjugated polyenes was constructed.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-DK-29009-13-LCP
Formerly
Z01-AM-29009-12-LCP

PERIOD COVERED
October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies on sickle cell disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI :	William Eaton	Medical Officer	LCP-NIDDK
OTHERS:	James Hofrichter	Research Chemist	LCP-NIDDK
	Andrea Mozzarelli	Guest Worker (Fogarty Fellow)	LCP-NIDDK
	P. San Biagio	Visiting Associate	LCP-NIDDK

COOPERATING UNITS *(if any)*

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Laboratory of Chemical Physics

SECTION
Macromolecular Biophysics

INSTITUTE AND LOCATION
NIH, NIDDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The mechanism of polymerization of hemoglobin S is being investigated in both purified solutions and single sickle cells in order to elucidate the molecular mechanism of this process. Theoretical analysis of kinetic data using both temperature jump and laser photolysis techniques shows that a double nucleation mechanism can quantitatively account for all of the major kinetic observations. Techniques are being developed to measure both the kinetics and thermodynamics of polymerization in single red cells at partial saturation with oxygen or carbon monoxide. These measurements will provide the most sensitive assay for testing the potential efficacy of therapeutic agents designed to inhibit intracellular polymerization in patients with sickle cell disease.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-DK-29010-14-LCP
Formerly
Z01-AM-29010-13-LCP

PERIOD COVERED
October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Conformation and electronic structure of biological molecules

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI :	William A. Eaton	Medical Officer	LCP-NIDDK
OTHERS:	James Hofrichter	Research Chemist	LCP-NIDDK
	Eric R. Henry	Senior Staff Fellow	LCP-NIDDK
	Lionel P. Murray	Staff Fellow	LCP-NIDDK

COOPERATING UNITS *(if any)*

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SECTION
Macromolecular Biophysics

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A transient laser spectrometer has been developed for measurements of optical absorption spectra and kinetics of hemoglobin conformational changes and ligand rebinding following photolysis of the carbon monoxide complex. Molecular dyanmics calculations are being carried out to simulate the heme and globin structural changes and the ligand motion following photolysis.

GPO 914-916

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-DK-29011-15-LCP
Formerly
Z01-AM-29011-14-LCP

PERIOD COVERED
October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The physics and chemistry of photoreception

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI :	William A. Hagins	Medical Officer	LCP-NIDDK
OTHERS:	S. Yoshikami	Research Biologist	LCP-NIDDK
	F. M. Hagins	Guest Worker	LCP-NIDDK
	M. C. Foster	Special Expert	LCP-NIDDK
	P. Rashidian	Electronics Engineer	LCP-NIDDK
	P. Ross	Research Chemist	LMB-NIDDK

COOPERATING UNITS *(if any)*

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SECTION
Section on Membrane Biophysics

INSTITUTE AND LOCATION
NIH, NIDDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	2	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An investigation of the mechanics of phototransduction in vertebrate photoreceptor cells.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-DK-29012-16-LCP
Formerly
Z01-AM-29012-15-LCP

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The influence of molecular structure on chemical and biological properties

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI :	Norman E. Sharpless	Research Chemist	LCP-NIDDK
Others:	Ralph G. Adams	Research Physicist	LCP-NIDDK
	William H. Jennings	Research Physicist	LCP-NIDDK

COOPERATING UNITS *(if any)*
Peter F. Kador, EI, LMOP, NIH
Frank Quinn, NCI
Jeffrey Gift, American University

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2	2	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Energy minimization calculations and quantum mechanical calculations on compounds of biological and pharmacological interest continue to give insights into and explanation of their modes of behavior, resulting in clues to their pharmacophores.

The inhibition of the enzyme aldose reductase by a wide variety of compounds continues under investigation by QSAR techniques, as well as by energy minimization computations, quantum mechanics and stereochemical considerations. A model for the interaction of a wide range of types of compounds with aldose reductase has been proposed, and their common features obtained.

Energy minimization and quantum calculations have been carried out on the various conformations of colchicine and isocolchicine to correlate binding properties with the energies and structures of their conformations.

Various compounds showing promise against the AIDS virus are being systematically investigated to obtain structural and electronic properties which may help elucidate the mechanism of their action, and thus lead to improved forms.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-DK-29015-15-LCP
Formerly
Z01-AM-29015-14-LCP

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Digital computer facilities for LCP and LMB

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

P.I. : W. H. Jennings, Jr. Research Physicist LCP-NIDDK

COOPERATING UNITS *(if any)*
Computer Systems Laboratory, DCRT: A. R. Schultz, Jr., J. I. Powell, D. C. Carpenter

LAB/BRANCH
Laboratory of Chemical Physics

SECTION
Section on Membrane Biophysics

INSTITUTE AND LOCATION
NIH, NIDDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The laboratory computer facility serving LCP and LMB consists of a host computer and a local network of twelve dedicated microcomputers serving laboratory instruments.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-DK-29016-11-LCP
Formerly
Z01-AM-29016-10-LCP

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Macromolecular dynamics and assembly reactions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. :	James Hofrichter	Research Chemist	LCP-NIDDK
OTHER:	William A. Eaton	Medical Officer	LCP-NIDDK
	Andrea Mozzarelli	Guest Researcher	LCP-NIDDK
	Eric Henry	Research Physicist	LCP-NIDDK
	Pier Luigi San Biagio	Guest Researcher	LCP-NIDDK
	Lionel Murray	Staff Fellow	LCP-NIDDK
	Zvi Kam	Guest Worker	LCP-NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Chemical Physics

SECTION
Spectroscopy and Structure

INSTITUTE AND LOCATION
NIH, NIDDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.2	2.2	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Transient spectroscopy is used to study the kinetics of conformational changes in macromolecules subsequent to excitation with a pulsed laser. Changes in both the tertiary and quaternary structure of hemoglobin have been observed following the photodissociation of carbon monoxide from the hemes.

Steady state photodissociation of carbon monoxide from hemoglobin S is used to study the thermodynamics and nucleation-controlled kinetics of the assembly of deoxyhemoglobin S into polymers. This technique has been used to study hemoglobin S in partially saturated solutions and to obtain delay times for solutions under physiological buffer conditions. Moreover, the kinetics of polymer formation can be monitored as the cell is being desaturated, permitting, for the first time, determination of the distribution of times required for cells to sickle at the saturations comparable to those of venous blood.

Quasi elastic light scattering is used to study the motion of deoxy hemoglobin S in solutions and gels.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-DK-29017-07-LCP
Formerly
Z01-AM-29017-06-LCP

PERIOD COVERED
October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Spectroscopic investigation of membrane lipids and models

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI :	Ralph G. Adams	Research Physicist	LCP-NIDDK
OTHERS:	Ira W. Levin	Research Chemist	LCP-NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Chemical Physics

SECTION
Section on Molecular Biophysics

INSTITUTE AND LOCATION
NIH, NIDDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Temperature programmed Raman spectroscopic studies comparing data from integrated intensity (II) with conventional peak intensity ratios and frequency shiftrs have demonstrated that II is much more sensitive to pretransitions, subtransiutions and changes in packing characteristics. In addition, it also indicates whether the packing is getting tighter or looser--something none of the other techniques can show. We have a new non-invasive very sensitive probe of intramembrane activities.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-DK-29019-06-LCP
Formerly
Z01-AM-29019-05-LCP

PERIOD COVERED
October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Theoretical studies on the dynamic aspects of macromolecular function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI :	A. Szabo	Research Chemist	LCP-NIDDK
Others:	G. Lamm	Staff Fellow	LCP-NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Chemical Physics

SECTION
Macromolecular Biophysics

INSTITUTE AND LOCATION
NIH, NIDDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	2	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

It is difficult to calculate the free energy of macromolecules from computer simulations because the entropy cannot be expressed as an equilibrium average. It is shown how one may systematically obtain successive approximations to the entropy using moments of the internal coordinate displacements evaluated from the simulation. A novel stochastic dynamics approach to the evaluation of the thermodynamic properties of quantum systems has been developed. This method is based on the similarity between the classical diffusion equation and the quantum mechanical Bloch equation for the density matrix. It is shown that for several systems, the results obtained using this approach are in good agreement with those based on the eigenvalues obtained by numerically solving the Schrodinger equation. A model for the influence of surface charge on the assembly of hemoglobin has been developed. In this model, the monomers first diffuse together, under the influence of their mutual electrostatic interaction, to form an encounter complex which then rearranges to form stereospecific contacts. This model quantitatively describes the measured association rate constants for the reaction between native α chains and a series of mutant β chains having surface charges that differ from the native by ± 1, ± 2 units.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-DK-29020-02-LCP
Formerly
Z01-AM-29020-01-LCP

PERIOD COVERED
October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Nuclear magnetic resonance: new methods and molecular structure determination

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI :	Ad Bax	Visiting Scientist	LCP-NIDDK
Others:	Edwin D. Becker	Research Chemist	LCP-NIDDK
	Rolf Tschudin	Electronics Engineer	LCP-NIDDK
	Laura Lerner	Arthritis Foundation Fellow	LCP-NIDDK
	Sankaran Subramanian	Visiting Scientist	LCP-NIDDK
	Vladimir Sklenar	Visiting Fellow	LCP-NIDDK
	Hong The Ha	Biological Laboratory Aid	LCP-NIDDK
	Lou Hughes	Guest Worker	LCP-NIDDK

COOPERATING UNITS *(if any)*
A. Aszalos, FDA; M.F. Summers, NCDB/FDA; G. Felsenfeld NIDDK/LMB; T.H. Mareci, Univ. Florida; L.G. Marzilli, Emory Univ.

LAB/BRANCH
Laboratory of Chemical Physics

SECTION
Section on Nuclear Magnetic Resonance

INSTITUTE AND LOCATION
NIH, NIDDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.8	4.5	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A new method has been developed that permits the regular ^{1}H spectrum of a molecule to be separated in a number of subspectra of limited fragments of the molecule. This simplifies spectral assignment and permits the exact measurement of coupling constants for spectral regions that were previously unaccessible. The method lengthens the apparent relaxation time of the nuclei during the mixing process which yields a large sensitivity enhancement in the study of macromolecules.

New two-dimensional NMR methods utilizing protons for indirect detection of nuclei with a low magnetogyric ratio (e.g. ^{13}C, ^{15}N) have been developed. These methods offer an increase in sensitivity of more than an order of magnitude relative to other modern NMR techniques. The methods can be used for detecting either direct (one-bond) connectivity or long range (multiple-bond) connectivity. This latter application has been shown to be extremely useful for structure determination of unknown compounds.

These new NMR techniques have been used to determine the structure of the antibiotic desertomycin and to reassign the spectrum of coenzyme B_{12}. The solution structure of coenzyme B_{12} has been studied by using spin-locked NOE spectroscopy.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DK-29021-01 LCP

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Confoormation and dynamics of biological macromolecules

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI :	Eric R. Henry	Research physicist	LCP-NIDDK
OTHER:	William A. Eaton	Medical officer	LCP-NIDDK

COOPERATING UNITS *(if any)*

Robin Hochstrasser, University of Pennsylvania

LAB/BRANCH

Laboratory of Chemical Physics

SECTION

Section on Macromolecular Biophysics

INSTITUTE AND LOCATION

NIH, NIDDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Molecular dynamics simulations are being carried out to aid in the interpretation of spectroscopic experiments on heme proteins. The dynamical fluorescence properties of tryptophans in myoglobin and the structural responses and thermal relaxation in heme proteins following photoexcitation and/or dissociation of bound ligands are being studied.

PHS 6040 (Rev. 1/84)

GPO 914-918

ANNUAL REPORT OF THE LABORATORY OF BIOORGANIC CHEMISTRY

NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

SECTION ON NEUROBIOLOGY

Receptors for Neurotransmitters and Drugs in Brain and Peripheral Tissues

We have previously demonstrated that exposure to a brief, ambient temperature swim elicited rapid and robust increases in [^{3}H]benzodiazepine binding in cerebral cortical membranes only in the presence of Eccles' permeable anions (e.g. chloride, iodide, bromide). These changes were manifest in increased apparent affinity (decreased K_d) of [^{3}H]flunitrazepam (the benzodiazepine used in this study) in the presence of these ions with no change in the maximum number of binding sites (B_{max}). No differences were observed in either basal or GABA-enhanced [^{3}H]benzodiazepine binding, which suggests that acute exposure to stress affected either the coupling between benzodiazepine receptors and chloride channels or chloride channels per se. The binding of [^{35}S]t-butylbicyclophosphorothionate [TBPS] (a "cage" convulsant thought to act at or near the benzodiazepine/GABA receptor coupled chloride channel) has now been studied in well-washed cortical membranes prepared from non-stressed and swim-stressed rats. Statistically significant increases in both the number of [^{35}S]TBPS binding sites and apparent affinity of this ligand were observed in cerebral cortical membranes prepared from swim-stressed rats. A more detailed investigation of this phenomenon revealed that the effects of stress were fully manifest after a four minute ambient temperature swim and were sustained during a thirty-minute swim. Alterations in the benzodiazepine/GABA receptor coupled chloride channel were also found in the hippocampus, a structure reported to play a critical role in the modulation of stress and anxiety. Stress induced changes in the chloride channel were also manifest as an increased potency and efficacy of Cl^- to enhance [^{35}S]TBPS binding and an increase in muscimol-enhanced [^{35}S]TBPS binding. These changes are postulated to represent a compensatory response of the organism to a stressful environment, since the effects of stress to augment Cl^--enhanced [^{3}H]flunitrazepam binding could be mimicked by the in vitro addition of pentobarbital, a drug which possesses anxiolytic and sedative actions, and which can directly increase chloride conductance as well as augment GABA-mediated chloride conductance.

Autoradiographic analysis of [^{35}S]TBPS binding in thin sections prepared from non-stressed and swim-stressed rats revealed significant changes in [^{35}S]TBPS binding in swim-stressed rats. These stress-induced changes were confined to specific areas of the cortex, amygdala, hippocampus, and cerebellum. The changes in radioligand binding in these specific brain areas appear far greater than those observed in broken-cell preparations. These studies represent the first attempt to "map" pathways which are involved in the response to stress or anxiety.

Since stress-induced changes in the benzodiazepine/GABA receptor coupled chloride channel were postulated to represent a compensatory change in this receptor-effector system to a stressor, we attempted to mimic the effects of stress on [^{35}S]TBPS binding by the in vitro application of a benzodiazepine. It has been previously reported that in vitro addition of a benzodiazepine to well-washed brain membranes has increased [^{35}S]TBPS binding, although the mechanism responsible for this phenomenon is unknown. We observed that a benzodiazepine (flunitrazepam) increased [^{35}S]TBPS binding in cortical membranes from both stressed and control animals in a concentration dependent fashion (maximum enhancement at ~100 nM). This effect was reversed by the addition of a benzodiazepine receptor antagonist, Ro 15-1788. However, the increase observed in non-stressed rats was significantly greater than in stressed rats. Scatchard analysis of this effect revealed that addition of flunitrazepam caused a significant reduction in the K_d of [^{35}S]TBPS with no change in the maximum number of binding sites. Thus, direct in vitro addition of a benzodiazepine partially mimicked the effects of stress on [35S]-TBPS binding, and this effect was significantly greater in tissue of animals not exposed to a prior stress.

The initial studies demonstrating rapid changes in the benzodiazepine/GABA receptor coupled chloride channel following ambient temperature swim stress were conducted with animals housed in a "conventional" animal facility. In order to determine whether this receptor-effector system was also subject to tonic control by the environment, both Cl^--enhanced [^{3}H]-flunitrazepam binding and [^{35}S]TBPS binding were studied in animals housed in a "protected" (low-stress) environment. Following a ten minute ambient temperature swim stress, animals maintained in both a protected environment and conventional facility had qualitatively similar increases in the number of [^{35}S]TBPS binding sites, the apparent affinity of this radioligand, and the efficacy of Cl^- to enhance [^{3}H]flunitrazepam binding. Nonetheless, the B_{max} of [^{35}S]TBPS and the efficacy of Cl^- to enhance [^{3}H]flunitrazepam binding were significantly lower in cortical tissue prepared from animals housed in the protected environment compared with animals housed in the conventional facility both before and after swim stress. Furthermore, sequential removal of rats from a common cage that were housed in a protected environment (cohort removal) produced a very rapid increase (<15 seconds) in Cl^- enhanced [^{3}H]flunitrazepam binding in cortical and hippocampal but not cerebellar membranes. Cohort removal also produced a sequential increase in the number of [^{35}S]TBPS binding sites and apparent affinity of this radioligand in cerebral cortical membranes. The effects of cohort removal were not observed if the animals were subjected to swim stress or removed from different cages. Measurement of stress-related hormones by radioimmunoassay (corticosterone, β-endorphin, ACTH, α-MSH) demonstrated that changes in the benzodiazepine/GABA receptor coupled chloride channel produced by cohort removal precede any statistically significant changes in circulating levels of these hormones. These findings demonstrate that the benzodiazepine/GABA receptor coupled chloride channel is under both tonic and acute regulation by the environment, and that this receptor-effector system may subserve a physiologically relevant function in the response to stressful or anxiety producing stimuli.

The "cage" convulsant [^{35}S]TBPS has been hypothesized to bind with high affinity to sites at or near a GABA-gated benzodiazepine receptor coupled chloride channel. Nonetheless, neither the precise location of these binding sites nor the biophysical significance of [^{35}S]TBPS binding are known. We compared the potencies of a series of anions in enhancing [^{35}S]TBPS binding with their relative permeabilities through GABA-gated chloride channels, and observed a high correlation ($r=0.9$) between these two parameters. Furthermore, statistically significant correlations ($r > 0.9$) were obtained between the K_d of [^{35}S]TBPS estimated in the presence of fixed concentrations of these anions and their relative permeabilities through GABA-gated chloride channels. The latter relationships obtained when correlations were estimated in either mouse spinal cord neurons or frog dorsal root ganglion cells. These findings strongly suggest that [^{35}S]TBPS binds directly to GABA-gated chloride channels, and that the apparent affinity of this radioligand is directly related to the permeability of these channels. Previous studies have demonstrated that the number of [^{35}S]TBPS binding sites is reduced in a concentration dependent fashion by incubation with phospholipase A_2 (PLA_2) (obtained from the venom of Naja naja). We subsequently observed that parallel reductions in pentobarbital and muscimol stimulated $^{36}Cl^-$ flux (uptake and efflux) are observed in synaptoneurosomes following incubation with this enzyme. These observations suggest that the number of [^{35}S]TBPS binding sites is related to the number of chloride channels in an "open" conformation. Thus, radioreceptor techniques using [^{35}S]TBPS may provide a simple means of describing the permeability characteristics and number of GABA-gated chloride channels.

Although previous studies suggested a relationship between halide-enhanced [^{3}H]benzodiazepine binding and the benzodiazepine/GABA receptor coupled chloride channel, no attempts have been made to evince a quantitative relationship between these parameters. Since we had shown that [^{35}S]TBPS binding is reduced in a concentration dependent fashion by phospholipase A_2 (PLA_2) while [^{3}H]benzodiazepine binding (in the absence of added ions or drugs) is unaffected by these concentrations of PLA_2, studies were conducted to determine whether a quantitative relationship exists between halide enhanced [^{3}H]benzodiazepine binding and [^{35}S]TBPS binding in rat cerebral cortex incubated with various concentrations of PLA_2. These studies revealed a quantitative relationship between the efficacy (i.e. maximum enhancement) of Cl^- to increase [^{3}H]flunitrazepam binding and the number of [^{35}S]TBPS binding sites in well-washed membrane fragments of rat cerebral cortex. This relationship (described by the equation $y=ab^x$) was maintained when [^{3}H]flunitrazepam binding was assayed in the presence of 100-600 mM Cl^-, and was not qualitatively altered by the presence of 100 µM pentobarbital. However, under experimental conditions that reduced the ratio of [^{35}S]TBPS binding sites/benzodiazepine receptors, the effects of pentobarbital suggest that the conductance state of the chloride channels may be the primary determinant of Cl^-enhanced [^{3}H]flunitrazepam binding.

The formulation of a model of the benzodiazepine/GABA receptor chloride ionophore complex in electrical rather than molecular terms resulted in the

prediciton that administration of a benzodiazepine receptor ligand with full "inverse agonist" (active antagonist) qualities could antagonize the pharmacologic actions of drugs that act to directly affect chloride currents, such as large doses of barbiturates. This hypothesis was tested by administering DMCM (6,7-dimethoxy-4-ethyl-3-carbomethoxy-β-carboline (1.5-15 mg/kg) to mice five minutes after injection of a lethal (LD_{94}) dose of pentobarbital. DMCM (1.5-5 mg/kg) increased short term survival (1 hour) in a dose dependent fashion, with an optimum survival rate more than 5 times greater than mice receiving pentobarbital alone. Higher doses also afforded an equivalent protection to the 5 mg/kg dose. A statistically significant increase was also observed in long term (24 hour) survival following both the 5 and 10 mg/kg dose of DMCM which was more than five times greater than in animals receiving pentobarbital alone. Divided doses of DMCM (5 and 2.5 mg/kg, respectively) administered 55 minutes apart increased 24 hour survival rates more than nine-fold compared to mice injected with pentobarbital alone. Statistically significant increases in 1 and 24 hour survival were produced by doses of DMCM which were not lethal when administered alone, and well below the CD_{50} of this compound. The protective effects of DMCM were blocked by pretreatment with the benzodiazepine receptor antagonist RO 15-1788, which suggests the effects of DMCM are mediated through the benzodiazepine receptor. These findings suggest that DMCM or another benzodiazepine receptor ligand with full "inverse agonist" qualities could prove effective as an antidote for barbiturate intoxication in man.

In a previous report, we described the quantitative measurement of $^{36}Cl^-$ efflux in "cell free" (synaptoneurosome) preparations of rat brain. This system was sensitive to both the effects of barbiturates and GABAmimetics, as well as GABA antagonists (e.g. bicuculline) and chloride channel blockers such as picrotoxin. The uptake of $^{36}Cl^-$ into synapto-neurosomes has now been demonstrated to be a sensitive measure of the effects of both barbiturates and GABAmimetics. Using this uptake system, it was demonstrated that ethanol (at concentrations present during acute intoxication (20-50 mM), stimulated Cl^- uptake in a concentration dependent fashion. The effects of ethanol were antagonized by bicuculline and picrotoxinin, and potentiated by pentobarbital and the GABAmimetic, muscimol. These findings may explain some of the pharmacological properties of ethanol and provides a mechanism for the common psychopharmacological actions of ethanol, barbiturates, and benzodiazepines.

6-Isothiocyano-3-carbomethoxy-β-carboline (NCS-β-CCM) inhibits the binding of [^{3}H]flunitrazepam and [^{3}H]Ro 15-1788 to "central" benzodiazepine receptors with an IC_{50} of ~2 nM in well washed membranes of rat cerebral cortex. This inhibition proved to be irreversible since extensive washing after a 20 min. preincubation with 20-100 nM of this compound did not diminish the effect of NCS-β-CCM. Comparable incubation of the parent compound β-CCM (IC_{50} ~1 nM) resulted in a loss of inhibition after two washes. The inhibition of [^{3}H]Ro 15-1788 produced by NCS-β-CCM was manifest as a change in the apparent K_d of this radioligand in both cortical and cerebellar membranes. In contrast, incubation of [^{3}H]flunitrazepam with low concentrations (20-50 nM) NCS-β-CCM produced a biphasic Scatchard plot, indicative of site heterogeneity, while incubation with higher concentrations of alkylator resulted in

a monophasic binding isotherm. These results suggest that NCS-β-CCM may preferentially alkylate a benzodiazepine receptor subtype, and should prove useful in the purification and isolation of benzodiazepine receptor subtypes.

In a previous report we demonstrated that injection of rats with the β-carboline FG 7142 (N-methyl-β-carboline-3-carboxamide) resulted in a significant reduction in mitogen (Concanavalin A and Phytohemagglutin A) stimulated T-cell proliferation 24 hours later, and that injection of the benzodiazepine receptor antagonist Ro 15-1788 reversed this effect. These findings suggested that chemically induced "anxiety" may profoundly suppress the immune response. We have extended these studies to a different species (mouse) and found a similar supression of mitogen stimulated T-cell proliferation 24 hours after either FG 7142 or DMCM. Cytotoxic T-lymphocyte (CTL) activity was also suppressed in these animals. A direct effect of these β-carbolines on the immune system as ruled out since in vitro addition of these compounds did not affect either mitogen-stimulated T-cell proliferation or CTL activity. The time-course for this β-carboline induced suppression of the immune response has now been studied at 1, 8, and 26 days after injection. Inhibition of mitogen-stimulated T-cell proliferation induced by these β-carbolines was not diminished at either 8 or 26 days after injection. The mechanism for this apparent enduring effect is under investigation, but could involve a "conditioning" effect of these β-carbolines.

A novel class of benzodiazepine receptor ligands has been synthesized by Dr. J. Cook and collaborators (Univ. of Wisconsin). These compounds (diindoles) are planar and (despite a structural dissimilarity) possess a charge distribution similar to another class of benzodiazepine receptor ligands, the pyrazoloquinolinones (represented by the benzodiazepine receptor antagonist CGS 8216). It has been shown that the parent molecule (MLT I-70) binds with high affinity to brain benzodiazepine receptors (K_i < 3 nM), and derivatives of MLT I-70 have affinities of 1-1200 nM. Since small substitution on the pyrazoloquinolinone ring results in a dramatic change in the pharmacologic properties of such (from a benzodiazepine antagonist to a benzodiazepine-like), in vivo testing is in progress to determine whether diindoles could represent a therapeutically useful class of compounds.

AHN 070, an isothiocynate derivative of the "peripheral" benzodiazepine receptor (PBR) ligand PK 11195 (an isoquinoline), was previously shown to bind irreversibly to PBR with an IC_{50} of ~20 nM. A complementary irreversible ligand derived from the prototype PBR ligand Ro 5-4864 has now been synthesized. This compound (AHN 086) contains an isothiocyanate moiety and binds irreversibly to PBR with an IC_{50} of ~1.3 nM. Using standard incubation conditions (50 mM potassium phosphate buffer, pH 7.0, 0°) AHN 086 reacted rapidly with PBR, whereas reduction of pH of the incubation mixture resulted in a time dependent inhibition of [^{3}H]Ro 5-4864 binding to PBR. These findings indicate a histidine residue is present at or near the active center of PBR. The specificity of AHN 086 to PBR was demonstrated by the failure of AHN 086 to inhibit [^{3}H]flunitrazepam binding to "central" benzodiazepine receptors (CBR) at concentrations of up to 1 μM. Thus, AHN 086 appears to be

a specific, high affinity irreversible ligand of PBR and should prove invaluable in purification and further characterization of this receptor site.

We have previously demonstrated that adrenalectomy produces a significant reduction in radioligand binding to renal PBR. This effect was reversed by administration of replacement doses of aldosterone, but not by administration of the glucocorticoid, dexamethasone. Administration of these compounds to sham operated animals did not affect the density of renal PBR. Similar effects of adrenalectomy were not observed in another tissue sensitive to aldosterone, the submandibular salivary gland. The decrease in renal PBR has now been localized to the renal cortex and the outer stripe of the medulla by gross dissection of renal slices and renal tissue section autoradiography. The specific effect of adrenalectomy on renal PBR density, the lack of direct effect of aldosterone of radioligand binding to PBR, and the localization of the changes in PBR density to the renal cortex suggests that these changes could reflect an adaptation of the renal nephron (possibly the distal convoluted tubule, intermediate tubule and/or collecting duct) to the loss of mineralocorticoid hormones.

Previous biochemical findings suggested that PBR in kidney could be involved in anion transport processes in the kidney. In order to further explore this possibility, rats were adminstered diuretic doses of furose-mide or hydrochlorothiazide for 3-5 days. These regimens produced significant increases in the density of renal PBR. A similar increase in density was also observed after rats were treated for one week with the prototype PBR ligand, Ro 5-4864. The increases in PBR density produced by Ro 5-4864 but not furosemide were prevented by co-administration of the PBR ligand, PK 11195. Further, administration of Ro 5-4864 increased 24 hour urine volume by ~135%. Pretreatment of animals with 0.1 mg/kg clonazepam blocker reduced the increase in 24 hour urine volume to ~70%, suggesting that some, but not all of the increase in urine volume could be attributed to the convulsant/anxiogenic effects of Ro 5-4864. Nonetheless, Na^+, K^+, and Cl^- concentrations were lower after Ro 5-4864, consistent with a direct diuretic action of this compound. These findings support previous observations from this laboratory which suggest that renal PBR may be associated with modulation of ion fluxes (K^+, Cl^-), and could be involved in the diuretic actions of some commonly used diuretic agents.

Previous studies from this laboratory have demonstrated a high density of PBR in rat pineal gland. The number of PBR in this tissue can be regulated (reduced) by exposure to constant light, superior cervical ganglionectomy, or administration of the adrenergic neurotoxin 6-hydroxydopamine. The characteristics of radioligand binding to PBR in the bovine pineal gland were examined in order to explore the feasibility of using this tissue as a model to study the relationship between PBR and the sympathetic nervous system. [^{3}H]PK 11195 binding to bovine pineal PBR revealed high affinity binding of this radioligand (K_d ~1.1 nM) to a population of sites with a density <5% of that reported in rat pineal gland (~0.8 versus ~26 pmol/mg protein). In contrast to rat pineal gland, no high affinity binding of [^{3}H]Ro 5-4864 was observed. Thus, bovine pineal PBR were further characterized using li-

gands structurally related to either Ro 5-4864 or PK 11195. The 2-fluoro isomer of PK 11195 (PK 11211) potently displaced [^{3}H]PK 11195 from bovine pineal membranes (K_i ~1.3 nM) while KW 1937 and triazolam (related to Ro 5-4864) displaced [^{3}H]PK 11195 with low affinities (K_i ~11.6 and 2.9 μM, respectively). Displacement of [^{3}H]PK 11195 from bovine PBR with Ro 5-4864 derivatives such as Ro 5-6900, KW 1976 or brotizolam revealed the presence of both high (K_i 229, 1152, and 686 nM, respectively) and low (K_i 5, 4.7 and 4.7 μM, respectively) affinity binding sites with a relative density of 4:1. These results indicate that although both bovine and rat pineal possess PBR, the pharmacological characteristics of bovine pineal PBR are markedly different from those previously described in the rodent.

A survey of [^{3}H] Ro 5-4864 binding to brain and peripheral tissue of rats exposed to either 80 five second inescapable tailshocks or no shock was conducted. This investigation revealed that inescapable tailshock stress caused a significant reduction in the density of [^{3}H]Ro 5-4864 binding to membranes from kidney (31%), cerebral cortex (29%), heart (19%) and pituitary (17%). However, no changes in [^{3}H]Ro 5-4864 binding were observed in hippocampal, lung, or adrenal membranes. Scatchard analysis of the [^{3}H]Ro 5-4864 binding to renal membranes revealed that these effects were due to a reduction in the density (B_{max}) of PBR with no change in the apparent affinity (K_d) of radioligands for these sites. A similar decrease in PBR density was observed using [^{3}H]PK 11195. Finally, the effects of a graded stress on [^{3}H] Ro 5-4864 binding was investigated using 5, 20, and 80 five-second inescapable shocks. In the kidney, 5 shocks significantly increased PBS density indicated by a 35% increase in [^{3}H]Ro 5-4864 binding, while 20 and 80 shocks caused a significant decrease in PBS density (22 and 31%, respectively). In cerebral cortex, 5 and 20 shocks had no effect on PBR density, while 80 shocks resulted in a significant (29%) decrease in density. These results suggest that PBR may be involved in the responses of both the CNS and peripheral tissues to stressful environments. These data are supported by the finding that [^{3}H]Ro 5-4864 to a significant decrease in the density of PBR was found in heart and kidney of the Maudsley Reactive Rat, which is bred for a high level of "fearfulness."

The effects of monovalent (Na^+, Li^+, K^+, Rb^+) and divalent (Ca^{2+}, Mg^{2+}, Mn^{2+}) cations on dihydropyridine calcium antagonist binding sites in brain and cardiac membranes were investigated using a low ionic strength buffer (5 mM Tris-HCl, pH 7.4), and the dihydropyridine, [^{3}H]nitrendipine. At 25°C, the monovalent cations Na^+, Li^+, and K^+ (100 mM) but not Rb^+ significantly decreased the apparent dissociation constant (Kd) but had no effect on the maximum binding site capacity (B_{max}) of [^{3}H]nitrendipine in brain. The divalent cations Ca^{2+}, Mg^{2+}, and Mn^{2+} (2 mM) significantly increased the B_{max}, but did not affect the K_d of [^{3}H]nitrendipine. The effects of cations were concentration dependent (EC_{50} monovalent cations 20-25 mM; EC_{50} divalent cations 50-200 μM) and demonstrated brain region selectivity. The effect of Ca^{2+}, but not Mg^{2+} or Mn^{2+} on [^{3}H]nitrendipine binding was described by a two site model. At 25°C, neither mono- or divalent cations altered the characteristics of [^{3}H]nitrendipine binding to rat cardiac membranes. At 37°C, Na^+ (100 mM) but not K^+ (100 mM) significantly increased the B_{max} of [^{3}H]nitren-

dipine in rat brain membranes. Ca^{2+} (2 mM) significantly increased the B_{max} of [^{3}H]nitrendipine binding to rat train membranes to a greater extent than at 25°C. Both Na^+ and K^+ had no effect on [^{3}H]nitrendipine binding to cardiac [^{3}H]nitrendipine. These observations suggest that the selective effects of mono- and divalent cations on [^{3}H]nitrendipine binding to rat brain and cardiac membranes may be associated with differences in the ability of dihydropyridine calcium antagonists to block calcium currents in brain and cardiac tissues.

Local anesthetics were also used to probe differences in the binding of [^{3}H]nitrendipine to dihydropyridine calcium antagonist binding sites in rat brain and cardiac membranes. Local anesthetics inhibited [^{3}H]nitrendipine binding to brain and cardiac membranes with the rank order of potency, dibucaine = proadifen >> tetracaine > meproadifen > S-RAC-109 > R-RAC-109 (R) > benzocaine. Lidocaine, procaine, piperocaine and bupiva-caine produced either a small potentiation or inhibition of [^{3}H]nitrendipine binding. Dibucaine inhibited [^{3}H]nitrendipine binding to brain membranes (IC_{50} 4.9 ± 0.5 µM) by increasing the K_d, while in cardiac membranes (IC_{50} 8.5 ± 0.9 µM), it both increased the K_d and decreased the B_{max} of [^{3}H]nitrendipine. The potency of dibucaine to inhibit [^{3}H]nitrendipine binding was reduced in both tissues by monovalent ($Li^+ > Na^+ = K^+ = Rb^+$; EC_{50} 40-50 mM) and divalent (Ca^{2+}, Mg^{2+} and Mn^{2+}; EC_{50} 10-50 µM) cations. These cations reduced the effect of dibucaine on the K_d of [^{3}H]nitrendipine in brain, and on the B_{max} of [^{3}H]nitrendipine in cardiac membranes. Inhibition of [^{3}H]nitrendipine binding by dibucaine was best described by high (2 µM) and low (50 µM) affinity sites. The apparent affinities of these sites, but not the fractional occupancies, were similar in brain and cardiac membranes. Na^+ modulated the occupancies of these sites in brain, but not in cardiac membranes, while Ca^{2+} inhibited occupancy of the high affinity site in both tissues. The effects of Li^+ were similar to those of Ca^{2+}. These findings indicate that brain and cardiac dihydropyridine calcium antagonist binding sites are coupled to different allosteric effectors or exist in a different membrane environment.

Phencyclidine was previously shown to allosterically increase the apparent affinity of the dihydropyridine ([^{3}H]nitrendipine) calcium antagonist binding site in a lysed synaptosomal membrane preparation of rat forebrain. Treatment of a similar preparation of mouse forebrain with 4-isothio-cyanato-1-(1-phenylcyclohexyl)piperidine (FOURPHIT), an acylating phencyclidine derivative, resulted in a concentration dependent (.1-10 µM), irreversible, increase in the apparent affinity of [^{3}H]nitrendipine. In contrast, the effects of phencyclidine were reversible. The FOURPHIT isomer, 1-[1-(3-isothiocyanatophenyl)cyclohexyl]piperidine (METAPHIT), (10 µM) also irreversibly increased the apparent affinity of [^{3}H]nitrendipine, but was much less efficacious than FOURPHIT. Phencyclidine blocked the irreversible increase in the apparent affinity of [^{3}H]nitrendipine produced by FOURPHIT. The interactions of multivalent cations and the calcium antagonist diltiazem with the [^{3}H]nitrendipine binding site were altered following treatment of membranes with FOURPHIT. These studies suggest that FOURPHIT irreversibly interacts with the same sites as PCP, and thus may be a useful tool with which

to further probe both the behavioral and biochemical interactions between phencyclidine and the dihydropyridine calcium antagonist binding sites.

SECTION ON OXIDATION MECHANISMS

Enzymatic Oxidation of Drugs to Toxic and Carcinogenic Metabolites

Previous annual reports from this Section have described a systematic approach to the study of the cytotoxicity, mutagenicity, and carcinogenicity of several polycyclic aromatic hydrocarbons. Briefly, these studies have consisted of i) synthesis of as many known and potential oxidative metabolites as was possible, ii) study of the metabolism of the hydrocarbons with these authentic standards in hand, iii) testing these compounds for cytotoxic and mutagenic activity with bacterial and mammalian cells both in the presence and in the absence of added drug metabolizing systems such as cytochrome P-450 and epoxide hydrolase, and iv) evaluation of the carcinogenicity of these synthetic standards in several animal models. These studies provided evidence which indicated that bay-region diol epoxides are the most potent carcinogenic metabolites of these hydrocarbons. We formulated the "bay-region" theory which predicts that diol epoxides that have the epoxide group in the bay region of the hydrocarbon will be the most chemically reactive and presumably biologically active diol epoxides from hydrocarbons that are tumorigenic. To date studies from our laboratory as well as several other laboratories around the world have either proved or implicated bay-region diol epoxides as ultimate carcinogens for benzo[a]pyrene, benz[a]anthracene, benz[c]acridine, 7-methylbenz[a]anthracene, 7-methylbenz[c]acridine, benzo-[b]fluoranthene, 7,12-dimethylbenz[a]anthracene, 3-methylcholanthrene, dibenz[a,h]anthracene, two dibenzpyrenes, chrysene, 5-methylchrysene, benzo[c]phenanthrene, and certain methylated cyclopentaphenanthrenes. The theory has stimulated considerable research in the field, all of which has supported our initial concepts. Significant exceptions to the theory have yet to emerge.

One aspect of hydrocarbon-induced carcinogenesis which the bay-region theory made no attempt to take into account is the relative and absolute stereochemistry of ultimate carcinogens. Many of our current efforts continue to address this question. Studies of the dihydrodiols and resultant bay-region diol epoxides formed from benz[a]anthracene as well as phenanthrene and chrysene by liver microsomes have shown that these molecules are all superimposable with the corresponding benzo[a]pyrene metabolites when their bay regions are aligned. In the benzo[a]pyrene case, only one of four stereoisomeric bay-region 7,8-diol-9,10-epoxides exhibits strong tumorigenic activity, namely the predominant metabolically formed isomer. Tumor studies have shown that the related stereoisomer (R,S-diol-S,R-epoxide) is also the active form from chrysene and benz[a]anthracene. Tumor studies on the optically active benzo[c]phenanthrene 3,4-diol-1,2-epoxides have now been completed. Results of the present studies are suggestive that there is a highly enantioselective site with which these carcinogens interact within the cell. Studies are in progress that will further define the steric constraints of the active site of cytochrome P-450c, the principal oxidative enzyme respon-

sible for the conversion of polycyclic aromatic hydrocarbons to ultimate carcinogens.

Chemistry of Polycyclic Aromatic Hydrocarbons. A major problem in this area has been our inability to utilize fully available NMR data for structural elucidation because of their complexity. This has now been circumvented with the description of a general procedure for the complete assignment of ^{1}H and ^{13}C NMR spectra of polycyclic hydrocarbons. It was shown that well-accepted two-dimensional NMR techniques (homo- and heteronuclear shift correlation) in combination with a recently developed one-dimensional NMR experiment for the determination of long-range heteronuclear connectivity provide sufficient information for complete and unambiguous ^{1}H and ^{13}C assignment of complex organic molecules. The procedure was illustrated for benzo[c]phenanthrene and its 5-bromo derivative, chrysene, and two benzochrysenes.

Because of our interests in the stereoselectivity of metabolism and expression of tumorigenic activity of the polycyclic hydrocarbons, new methods have been developed for the synthesis of optically pure metabolites of known absolute configuration. Synthesis of enantiomerically pure benzo[c]phenanthrene (+)-(5S,6R)- and (-)-(5R,6S)-oxides was described from diastereomerically pure (-)-(5R,6R)-trans-5-bromo-6-[(menthyloxy)acetyl]-and (+)-(5S,6S)-trans-5-bromo-6-[(menthyloxy)acetyl]-5,6-dihydrobenzo(c)phenanthrene derived from (-)-(menthyloxy)acetic acid. Configurational assignment of the enantiomeric arene oxides was based on correlation of the CD spectra of their trans-N-acetyl-L-cysteine adducts as methyl esters with the bis((-)-α-methoxy(trifluoromethyl)phenylacetate) of (+)-(5R,6R)-trans-5,6-dihydroxy-5,6-dihydrobenzo[c]phenanthrene of known absolute configuration. Separable major and minor S adducts were obtained from each arene oxide enantiomer. Structures of the major (attack at C-6) and minor (attack at C-5) adducts were established through the use of 5-deuterated arene oxide. Predominant attack (3:1) of the thiolate at C-6 of the arene oxide is consistent with PMO calculations.

K-region trans dihydrodiols of benzo[c]phenanthrene, chrysene, pyrene, and dibenz[c,h]acridine have been resolved as their diastereomeric diesters with (-)-(menthyloxy)acetic acid, and their absolute configurations have been assigned by the application of circular dichroism and exciton chirality methods. For these as well as the K-region trans dihydrodiol derivatives from five other hydrocarbons, a consistent pattern of physical properties has emerged. The R,R diastereomers are less retained on silica gel HPLC columns when eluted with ether/cyclohexane mixtures and show negative values of $[\alpha]_D$ in tetrahydrofuran, the degree of magnetic nonequivalence between H_A and H_B in the $-OCH_AH_BCO_2-$ portion of the diesters (100 MHz, C_6D_6) is generally much higher for the S,S enantiomers of the dihydrodiols, and the free R,R dihydrodiols have positive values of $[\alpha]_D$ in tetrahydrofuran provided their hydroxyl groups do not have a marked preference for the pseudodiaxial conformation.

Metabolism of Polycyclic Aromatic Hydrocarbons. Over the past several years, we have been in the process of refining a steric model for the cata-

lytic binding site of cytochrome P450c, the most effective cytochrome P450 isozyme known for metabolism of the polycyclic aromatic hydrocarbons. The absolute configurations of the enantiomeric 5,6-arene oxides of 7,12-dimethylbenz[a]anthracene (DMBA) had been recently assigned such that the late eluting enantiomer from a chiral HPLC column was assigned 5R,6S absolute configuration. The authors of that study further concluded that the 5R,6S-enantiomer predominated on metabolism of DMBA by cytochrome P450c in liver microsomes from 3-methylcholanthrene-treated rats. Their chemical assignment of absolute configuration has now been shown by us to be incorrect. Thus, metabolism of DMBA by these microsomes as well as by homogeneous cytochrome P450c produces 5,6-oxide highly enriched (95%) in the 5S,6R-enantiomer in accord with theoretical predictions of our model.

Metabolism of (+)-, (-)-, and (±)-trans-3,4-dihydroxy-3,4-dihydrobenzo-[c]phenanthrenes by liver microsomes from rats and mice and by a purified monooxygenase system reconstituted with cytochrome P-450c has been examined. Bay-region 3,4-diol 1,2-epoxides are minor metabolites of both enantiomers of the 3,4-dihydrodiol with liver microsomes from 3-methylcholanthrene-treated rats or with the reconsituted system (<10% of total metabolites). Microsomes from control and phenobarbital-treated rats and from control mice form higher percentages of these diol epoxides (13-36% of total metabolites). Microsomes from 3-methylcholanthrene-treated rats and cytochrome P-450c in the reconstituted system form exclusively the diol exposide-1 diastereomer, in which the benzylic hydroxyl group and oxirane oxygen are cis to each other, from the (+)-(3S,4S)-dihydrodiol. The same enzymes selectively form the diol expoxide-2 diastereomer, with its oxirane oxygen and benzylic hydroxyl groups trans to each other, from the (-)-(3R,4R)-dihydrodiol (77% of the total diol epoxides). Liver microsomes from control rats show similar stereoselectivity whereas liver microsomes from phenobarbital-treated rats and from control mice are less stereoselective. Three bis-dihydrodiols and three phenolic dihydrodiols are also formed from the enantiomeric 3,4-dihydrodiols of benzo[c]phenanthrene. A single diastereomer of one of these bis-dihydrodiols with the newly introduced dihydrodiol group at the 7,8-position accounts for 79-88% of the total metabolites of the (-)-(3R,4R)-dihydrodiol formed by liver microsomes from 3-methylcholanthrene-treated rats or by the reconstituted system containing epoxide hydrolase. In contrast, the (+)-3S,4S)-dihydrodiol is metabolized to two diastereomers of this bis-dihydrodiol, a third bis-dihydrodiol, and two phenolic dihydrodiols. The low percentage of diol epoxides formed with cytochrome P450c is consistent with our site model for this enzyme and may be in part responsible for the low tumorigenicity of the parent hydrocarbon.

Biological Activity of Polycyclic Hydrocarbon Metabolites. The present report completes our studies on the activity of benzo[c]phenanthrene metabolites. Tumorigenic activities of the (+)- and (-)-enantiomers of the diastereomeric, bay-region benzo[c]phenanthrene 3,4-diol-1,2-epoxides were evaluated in two mouse tumor models. In an initiation-promotion experiment on mouse skin, a single topical application of 10, 25, or 75 nmol of the compounds was followed by 20 weeks of promotion with 12-O-tetradecanoylphorbol-13-acetate. Of the four optical isomers of the bay-region diol epoxides,

(-)-(1R,2S,3S,4R)-3,4-dihydroxy-1,2-epoxy-1,2,3,4-tetrahydrobenzo[c]phenanthrene [(-)-diol epoxide-2] and (+)-(1R,2S,3R,4S)-3,4-dihydroxy-1,2-epoxy-1,2,3,4-tetrahydrobenzo[c]phenanthrene [(+)-diol epoxide-1] had equally high tumor-initiating activity while (+)-[1S,2R,3R,4S]-3,4-dihydroxy-1,2-epoxy-1,2,3,4-tetrahydrobenzo[c]phenanthrene [(+)-diol epoxide-2] had less than one-half of the activity of (-)-diol epoxide-2 and (+)-diol epoxide-1. (-)-(1S,2R,3S,4R)-3,4-Dihydroxy-1,2-epoxy-1,2,3,4-tetrahydrobenzo[c]phenanthrene [(-)-diol epoxide-1] was inactive at the doses tested. In newborn mice, (-)-diol epoxide-2 was almost 10-fold more active in producing lung tumors (average number of lung tumors/mouse) than the next most active compound, (+)-diol epoxide-2, at a total dose of 10 nmol. The enantiomers of diol epoxide-1 were inactive at this dose. When the total dose of each optical isomer was increased to 50 nmol, (-)-diol epoxide-1 was still inactive, and (+)-diolepoxide-1 produced a significant number of lung tumors (0.9 lung tumor/mouse), but this isomer still had less than 10% of the activity of the (+)- and (-)-diol epoxide-2 isomers. (-)-Diol epoxide-2, but none of the other optical isomers, also produced a significant incidence of hepatic tumors at the higher dose, and this compound was found to be the most tumorigenic bay-region diol epoxide ever tested in newborn mice. Racemic diol epoxide-1 had approximately 1% of the tumorigenic activity of racemic diol epoxide-2 in newborn mice, but both rademates had equal tumor-initiating activity on mouse skin. These results dramatically illustrate the complexities involved in ranking the relative tumorigenic activities of compounds in different tumor models.

In the previous report we described the synthesis of a number of dibenz[c,h]acridine derivatives which were to be tested for biological activity. The mutagenic activities of the enantiomers of the diastereomeric pair of bay-region 3,4-diol-1,2-epoxides have been evaluated in histidine-dependent strains of Salmonella typhimurium and in an 8-azaguanine-sensitive line of Chinese hamster cells. In strains TA 98 and TA 100 of Salmonella typhimurium the pair of enantiomers with (1R,2S,3S,4R) and (1S,2R,3R,4S) absolute configuration and the benzylic hydroxyl group trans to the epoxide oxygen are 2 to 4 times more mutagenic than the (1S,2R,3S,4R) and (1R,2S,3R,4S) isomers in which the benzylic hydroxyl and epoxide oxygen are cis. In both strains of bacteria there is very little difference in mutagenic activity between the enantiomers of each diastereomer. In contrast to these results in bacteria, the bay-region 3,4-diol-1,2-epoxide isomer with (1R,2S,3S,4R) absolute configuration is 5 to 7 times more mutagenic to Chinese hamster V79 cells than the other 3 isomers. The enantiomeric pair of bay-region tetrahydro-1,2-epoxides of dibenz[c,h]acridine are at least 7 times more mutagenic than the diol epoxides in the Salmonella assay, and no difference in mutagenic activity is observed between enantiomers. In the Chinese hamster V79 cell system, however, the tetrahydro-1,2-epoxide with (1R,2S) absolute configuration is 2- to 3-fold more mutagenic than its enantiomer with (1S,2R) absolute configuration. Results of metabolic activation experiments with the bacterial mutagenesis system and microsomes from Aroclor 1254-treated rats are consistent with the mutagenicity data described above, and support the concept that dibenz[c,h]acridine is metabolically activated to a bay-region diol epoxide. Notably, i) 3,4-dihydrodibenz[c,h]acridine, the expected precursor of a bay-

region tetrahydroepoxide, is metabolized to a potent mutagen, ii) racemic dibenz[c,h]acridine 3,4-dihydrodiol is metabolized to products which are several-fold more mutagenic than products of metabolism of dibenz[c,h]acridine or its 1,2- or 5,6-dihydrodiols, and iii) the tetrahydro 3,4-diol which lacks the isolated bay-region double bond is not metabolically activated to a bacterial mutagen.

Inhibition of Tumorigenic Activity. Ellagic acid, quercetin and robinetin were tested for their ability to antagonize the tumor-initiating activity of benzo[a]pyrene (B[a]P) and (±)-7β,8α-dihydroxy-9α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (B[a]P 7,8-diol-9,10-epoxide-2), the ultimate carcinogenic metabolite of benzo[a]pyrene. Ellagic acid, robinetin or quercetin (2500 nmol) had no tumor-initiating activity on mouse skin, but the topical application of 2500 nmol of ellagic acid 5 min before a tumor-initiating dose of 200 nmol of B[a]P 7,8-diol-9,10-epoxide-2 caused a 59-66% inhibition in the number of skin tumors per mouse that were observed after 15-20 weeks of promotion with 12-O-tetradecanoylphorbol-13-acetate. Similar treatment with 2500 nmol of robinetin or quercetin caused a statistically insignificant 16-24% inhibition in the tumor-initiating activity of 200 nmol of B[a]P 7,8-diol-9,10-epoxide-2 applied 5 min later. Treatment of mice with 2500 nmol of ellagic acid 5 min before the application of 50 nmol of B[a]P inhibited the mean number of skin tumors per mouse by 28-33% after 15-20 weeks of promotion, but these decreases were not statistically significant. Robinetin and quercetin had little or no effect on the tumor-initiating activity of B[a]P on mouse skin. Treatment of preweanling mice with 1/7, 2/7 and 4/7 of the total dose of ellagic acid (300 nmol), robinetin (1400 nmol), myricetin (1400 nmol) or quercetin (1400 nmol) i.p. on their first, eighth and fifteenth day of life, respectively, did not cause the formation of tumors in animals that were killed 9-11 months later. Similar treatment of preweanling mice with the above doses of the phenolic compounds 10 min before the i.p. injection of a total dose of 30 nmol of B[a]P 7,8-diol-9,10-epoxide-2 during the animal's first 15 days of life caused a 44-75% inhibition in the number of diol-epoxide-induced pulmonary tumors per mouse. Similar treatment with these plant phenols had little or no effect on B[a]P-induced pulmonary tumors.

The 12 isomeric phenols of benzo[a]pyrene were tested for their ability to inhibit the mutagenic activity of B[a]P 7,8-diol-9,10-epoxide-2], 3-Hydroxybenzo[a]pyrene (3-HO-B[a]P), a major metabolite of benzo[a]pyrene, was the most potent antagonist tested. Approximately 3 nmol of 3-HO-B[a]P, 14 nmol of 10-HO-B[a]P, and 5-8 nmol of 1-, 2-, 4-, 5-, 6-, 7-, 8-, 9-, 11-, and 12-HO-B[a]P inhibited the mutagenic activity of 0.05 nmol of B[a]P 7,8-diol-9,10-epoxide-2 by 50% in Salmonella typhumurium strain TA 100. The importance of the phenolic group for antimutagenic activity was indicated by the lack of antimutagenic activity of benzo[a]pyrene itself. 3-HO-B[a]P also inhibited the mutagenic activity resulting from the metabolic activation of benzo[a]pyrene and (±)-trans-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene by rat liver microsomes. This inhibition may have resulted from an effect of 3-HO-B[a]P on the metabolic activation of these carcinogens and/or from a direct effect on the action of B[a]P 7,8-diol-9,10-epoxide-2. In a mammalian cell

culture system utilizing Chinese hamster V79 cells, 3-HO-B[a]P (8 μM) inhibited the mutagenicity of B[a]P 7,8-diol-9,10-epoxide-2 (0.1 μM) by 50%. Although 3-HO-B[a]P was a potent inhibitor of the mutagenic activity of bay-region diol epoxides of benzo[a]pyrene, dibenzo[a,h]pyrene, and dibenzo[a,i]-pyrene in S. typhimurium strain TA 100, higher concentrations of 3-HO-B[a]P were needed to inhibit the mutagenicity of the chemically less reactive benzo[a]pyrene 4,5-oxide and the bay-region diol epoxides of benz[a]anthracene, chrysene, and benzo[c]phenanthrene.

Both 3-HO-B[a]P and 10-HO-B[a]P accelerated the disappearance of B[a]P 7,8-diol-9,10-epoxide-2 from 1:9 dioxane-water solutions at pH 7 and 25°C. 3-HO-B[a]P, the most effective antimutagen of the B[a]P phenols tested, was much more reactive with the diol epoxide than 10-HO-B[a]P, the least effective antimutagen. The rate constant for the reaction of 3-HO-B[a]P with the diol epoxide exhibited a nonlinear (>first-order) dependence on the concentration of the phenol. Evidence was obtained for covalent adduct formation between the diol epoxide and each of the two phenols. A 3-HO-B[a]P adduct was isolated and characterized spectroscopically as the adduct derived from cis addition of the 3-hydroxyl group of 3-HO-B[a]P to the C-10 position of B[a]P 7,8-diol-9,10-epoxide-2.

Although a 2500 gnmol dose of 3-HO-B[a]P had weak tumor-initiating activity on mouse skin, the topical application of this amount of the phenol 5 min before a tumor-initiating dose of 200 nmol of B[a]P 7,8-diol-9,10-epoxide-2 caused a more than 70% decrease in the number of diol epoxide-induced skin tumors that were observed after 16-20 weeks of promotion with 12-O-tetradecanoylphorbol-13-acetate. This dosing regimen of 3-HO-B[a]P had little or no effect on the tumor-initiating activity of 50 nmol of B[a]P, but the application of 2500 nmol of 3-HO-B[a]P 5 min before and 60 min after a 50-nmol initiating dose of B[a]P caused a modest inhibition in the mean number of B[a]P-induced skin tumors.

Cholesterol Oxide Hydrolase. Immunochemical studies in the previous annual report established this enzyme activity as new form of microsomal epoxide hydrolase. Cholestane 3β,5α,6β-triol has been identified as the exclusive product formed on hydration of cholesterol 5,6α- and 5,6β-oxide catalyzed by cholesterol oxide hydrolase in liver microsomes obtained from five mammalian species. Highest activities were present in microsomes from rats and humans. Both acid- and base-catalyzed hydrolysis of the two epoxides also produce this product, presumably due to preference for pseudoaxial opening of the oxirane ring to form product with a trans-AB ring junction. Although the β-oxide is more reactive than the α-oxide upon acid-catalyzed hydration, the α-oxide is a 4.5-fold better substrate than the β-oxide as indicated by values of V_{max}/K_m. The kinetic parameters V_{max} and K_m for the reaction catalyzed by rat liver microsomes are $1.68 \pm 0.15 \times 10^{-7}$ M min^{-1} and 10.6 ± 1.5 μM for the α-oxide and $1.32 \pm 0.11 \times 10^{-7}$ M min^{-1} and 37.2 ± 5.5 μM for the β-oxide at 0.35 mg protein/ml, pH 7.4, 6.35% (v/v) CH_3CN and 37°C. Several imino compounds are competitive inhibitors for the enzyme from rat liver. The most effective of these is 5,6α-iminocholestanol (K_i = 0.085 μM) which was known to be a good inhibitor from previous studies. Inhibition by

aziridines is consistent with the participation of acid catalysis in the mechanism of action of the enzyme. Cholesterol oxide hydrolase is a distinct enzyme from oxidosqualene cyclase as well as microsomal epoxide hydrolase (EC 3.3.2.3) and the recently reported mouse hepatic microsomal epoxide hydrolase that catalyzes the hydration of trans-stilbene oxide.

7-Dehydrocholesterol 5,6β-oxide covalently modifies and inactivates the rat liver microsomal enzyme cholesterol oxide hydrolase. The covalent modification is presumed to occur at the active site of the enzyme since 5,6α-iminocholestanol, a potent competitive inhibitor of the enzyme, blocks incorporation of 3-[^{3}H]-7-dehydrocholesterol 5,6β-oxide into the protein. Kinetics of the inactivation were measured both by following the loss of catalytic activity and by monitoring incorporation of 3-[^{3}H]-7-dehydrocholesterol 5,6β-oxide into microsomal protein. Both the loss of catalytic activity and the incorporation of label followed first order kinetics. Linear plots of the reciprocal of the pseudo-first order rate constants for the loss of catalytic activity and for the incorporation of radioactivity versus reciprocal of inhibitor concentrations indicated saturation kinetics. The kinetic parameter k_{inac} was found to be $(2.83 \pm 0.43) \times 10^{-3}$ s^{-1} measured either by incorporation of tritium (300 mM potassium phosphate buffer, pH 8.0, 2.4 mg of microsomal protein/ml at 37°C) or by the loss of catalytic activity (300 mM potassium phosphate buffer, pH 7.5, 0.99 mg of microsomal protein/ml at 37°C). Unlike xenobiotic microsomal epoxide hydrolase (EC 3.3.2.3) which is not inactivated or inhibited by 7-dehydrocholesterol 5,6β-oxide, cholesterol oxide hydrolase appears to hydrolyze cholesterol oxides via a positively charged transition state.

SECTION ON PHARMACODYNAMICS

Cyclic Nucleotides and Other Second Messengers in the Nervous System

Adenosine Receptors: Structural Analysis of Agonist Activity. A series of over one hundred and fifty adenosine analogs have been investigated with respect to i) inhibition of binding of [^{3}H]cyclohexyl adenosine to an A_1-adenosine receptor in rat brain membranes, ii) inhibition of adenylate cyclase via an A_1-adenosine receptor in rat fat cell membranes, iii) stimulation of adenylate cyclase in membranes via A_2-adenosine receptors from rat pheochromocytoma PC12 cells and human platelets and iv) stimulation of coronary blood flow in dog via an A_2-adenosine receptor. The A_1 receptors show greater stereoselectivity in the N^6 region of the receptor towards assymmetric aralkyl substituents than do the A_2 receptors. The A_1 receptors show greater bulk tolerance in the N^6 region such that they retain affinity for certain N^6-tertiary alkyladenosines and N^6-cycloalkyladenosines that have low activity at A_2 receptors in platelets and PC12 cells and are inactive at the coronary A_2 receptor. At the coronary A_1 receptor, the most potent analogs have either aliphatic N^6-substituents with four or more methylene residues or have an N^6-halophenyl subsitutent. There is a strong correspondance in agonist activity profiles at the two A_1 receptors. At the coronary A_2 receptor, the most potent analogs have an N^6-phenethyl or similar heteroarylethyl substituent. Significant differences in the agonist profiles of the two A_2

receptors ccoupled to cyclase and at the coronary A_2 receptor indicate structural heterogeneity within the members of this adenosine receptor subclass. The lack of correspondence between the structure-activity relationships of these analogs at the A_1 and A_2 receptors appear definitive in terms of establishing the existence of A_1 and A_2 subclasses of adenosine receptors. A set of adenosine analogs including several N^6-substituted analogs, such as N^6-cyclohexyl-, N^6-R- and S-1-phenyl-2-propyladenosines, 5'-N-ethylcarboxamidoadenosine and its N^6-cyclohexyl derivative, 2-chloroadenosine and 2-phenylaminoadenosine appear to represent a series on analogs sufficient for characterization of A_1 and A_2 classes of adenosine receptors.

Adenosine Receptors: Structural Analysis of Antagonist Activity for Caffeine and Theophylline Derivatives. A variety of analogs of caffeine and theophylline in which the 1-, 3-, and 7-methyl substituents have been replaced with n-propyl, allyl, propargyl, and i-butyl were investigated at A_1-and A_2-adenosine receptors in rodent brain tissue. Caffeine and theophylline are nonselective for these receptors. Nearly all of the twenty-two analogs of caffeine are more potent than caffeine itself at adenosine receptors. Replacement of the 1-methyl moiety with n-propyl, allyl or propargyl substituent has little effect on potency at the A_1 receptor, while enhancing potency about 7 to 10-fold at the A_2 receptor; 3,7-Dimethyl-1-propylxanthine is only slightly (1.4-fold) more potent than caffeine at the A_1 receptor, while being 10-fold more potent at the A_2 receptor. 1,3-Di-n-propyl-7-methylxanthine is also selective for the A_2 receptor, being 8-fold more potent than caffeine at the A_1 receptor and 40-fold more potent at the A_2 receptor. A number of other caffeine analogs including 3,7-dimethyl-1-n-propylxanthine, 7-allyl-1,3-dimethylxanthine, and 1,3-dimethyl-7-propargylxanthine are also somewhat selective for the A_2 receptor. The most potent caffeine analog was 1,3-di-n-propyl-7-propargylxanthine, which was about 100-fold more potent than caffeine at both A_1 and A_2 receptors. The theophylline analogs were relatively nonselective except for a 1-ethyl analog and the 1,3-di-n-propyl, 1,3-di-i-butyl and 1,3-dibenzyl analogs, which were somewhat selective for the A_1 receptor. 1,3-Di-n-propylxanthine was 20-fold more potent than theophylline at the A_1 receptor and 5-fold more potent at the A_2 receptor. Several analogs were investigated further as antagonists at A_2 adenosine receptors stimulatory to adenylate cyclase in membranes from rat pheochromocytoma PC12 cells and human platelets and at A_1 adenosine receptors inhibitory to adenylate cyclase from rat fat cells. Among these analogs, 1-propargyl-3,7-dimethylxanthine was about 4- to 7-fold and 7-propyl-1,3-dimethylxanthine about 3- to 4-fold more potent than caffeine at A_2 receptors of PC12 cells and platelets. At A_1 receptors of fat cells, both compounds were about 2-fold less potent than caffeine. These caffeine analogs have an A_1/A_2 selectivity ratio of about 10-20 and are the first selective A_2 receptor antagonists yet reported. The results may provide the basis for the further development of highly potent and highly selective A_2 adenosine receptor antagonists.

Adenosine Receptors: Structural Analysis of Antagonist Activity for 8-phenyl-1,3-dialkylxanthine. The effect of a variety of aryl substituents on the potency and selectivity of nineteen analogs of 1,3-dipropyl-8-phenyl-

xanthine as antagonists at A_1- and A_2-adenosine receptors in brain tissue were determined. The 4-sulfonamidophenyl- and 4-carboxamidophenyl analogs are potent and somewhat selective for the A_1 receptor. None of the dihydroxyphenyl analogs are remarkably potent, but all are selective for the A_1 receptor. 1,3-Dipropyl-8-(2-hydroxy-4-methoxyphenyl)xanthine is the most selective A_1 antagonist of the analogs with an A_1/A_2 potency ratio of about 90. A variety of functionalized congeners of 8-phenyltheophylline and 1,3-dipropyl-8-phenylxanthine were developed as radioligands and potent and selective probes for adenosine receptors. Certain of these functionalized congeners, which contain a functionalized chain used for covalent attachment to amines, amino acids, oligopeptides, fluorophores and spin labels, have proven useful probes for A_1 and A_2 receptors.

Second Messengers, Interrelationships of Cyclic AMP Generation and Phosphatidylinositol Breakdown. The protein kinase C activator, phorbol-12-myristate-13-acetate (PMA), augments the cyclic AMP accumulation induced by forskolin in pheochromocytoma (PC12) cells with an EC_{50} value of 14 nM, while having no effect on basal values. At a concentration of 100 nM PMA markedly augmented the magnitude of the forskolin response and, in addition, caused a slight increase in the potency of forskolin. PMA also enhanced the maximal cyclic AMP accumulation produced by 2-chloroadeno-sine, and caused a slight increase in potency of the adenosine analog. Since PMA mimics the effect of diacyglycerols that form during the turnover of the membrane lipid phosphatidylinositol, the results suggest an interrelationship between the systems involved in phosphatidylinositol turnover and cyclic AMP generation in PC12 cells.

Second Messengers. Stimulation of Accumulations of Cyclic AMP and Inositol Phosphates by Agents that Increase Influx of Sodium Ions in Brain Preparations. Activation of α_1-adrenergic receptors by norepinephrine in guinea pig cortical synaptoneurosomes augments accumulations of cyclic AMP elicited by 2-chloroadenosine and concomitantly increases formation of inositol phosphates. Various agents that affect calcium channels or sites of action of calcium have little or no effect on cyclic AMP accumulation elicited either with 2-chloroadenosine, or with a 2-chloroadenosine, or with a 2-chloroadenosine/norepinephrine combination, nor did they markedly affect formation of inositol phosphates elicited by norepinephrine. However, EGTA reduces both cyclic AMP accumulation and inositol phosphate formation. Agents such as batrachotoxin, veratridine, aconitine, scorpion toxins, pumiliotoxin B and certain pyrethroids that are active at voltage-dependent sodium channels enhance accumulations of cyclic AMP and inositol phosphates. Ouabain, an agent that will increase accumulation of internal sodium by inhibition of sodium-potassium ATPase, also stimulates formation of [^{3}H]insositol phosphates as does monensin, a sodium ionophore. Tetrodotoxin and saxitoxin, specific blockers of voltage-dependent sodium channels, prevent or reduce the stimulatory effects of sodium channel agents and ouabain on phosphatidylinositol turnover, while having lesser or no effect, respectively, on receptor-mediated or monensin-mediated stimulation. Removal of extracellular sodium ions markedly reduces stimulatory effects of a sodium channel agent, while removal of extracellular calcium ions with EGTA blocks both receptor-

mediated and sodium channel agent-mediated phosphatidylinositol turnover. Calcium channel blockers have little or no effect on responses to such agents. The results provide evidence for a hitherto unsuspected messenger role for sodium ions in excitable tissue, whereby neuronal activity and the resultant influx of sodium will cause activation of phospholipase systems involved in hydrolysis of phosphatidylinositols, thereby generating two second messengers, i) the inositol phosphates, which mobilize calcium form internal stores, and ii) the diacylglycerols, which activate protein kinase C. It is proposed that enhanced influx of sodium ions increases phosphatidylinositol metabolism, resulting in formation of diacylglycerols and inositol phosphates, and that the former, through activation of protein kinase, causes an enhancement of cyclic AMP accumulations in brain tissue.

Physiological Role for Cyclic AMP in Desensitization. Forskolin, an activator of adenylate cyclase, and its analogs were studied on the nicotinic acetylcholine receptor-ion channel complex (AChR) of rat and frog skeletal muscles. At nanomolar concentrations, forskolin caused desensitization of the AChR. The ability of forskolin and its analogs to desen-sitize the nicotinic AChR and to activate adenylate cyclase were strongly correlated, indicating an involvement of phosphorylation of AChR via cyclic AMP on the desensitization process.

SECTION ON PHARMACODYNAMICS

Pharmacologically Active Compounds from Amphibians and Other Natural Sources

New Alkaloids from Poison Frogs. Forty alkaloids were detected and characterized for skin extracts of two populations of the poison frog Dendrobates histrionicus from northwestern Colombia. Combined gas chromatography-mass spectrometry with ammonia or deuteroammonia in a chemical ionization mode detected protonated parent ions and determined the number of exchangeable NH and OH hydrogens. Six previously unknown dendrobatid alkaloids were characterized. Two were 2,5-disubstituted pyrrolidines, which included pyrrolidine 197B, a trans-2-butyl-5-pentylpyrrolidine, while a third was a 2,6-dipentylpiperidine. Indolizidines 239AB and 239CD had the same relative configuration as the parent alkaloid 223AB [(5E,9E)3-butyl-5-propylindolizidine] and contained, respectively, a ω-hydroxy group in the propyl or butyl side chain. The profiles of alkaloids in the new northern populations of D. histrionicus are typical of the species in containing a set of about eight histrionicotoxins, in marked contrast to a related species, D. lehmanni, which does not contain histrionicotoxins. Skin extracts, from the Panamanian frog Dendrobates speciosus contain a variety of indolizidines and decahydroquinolines. Isomers of pumilotoxins B and 267A were characterized along with a 4-hydroxy-2-methyl-6-decylpiperidine alkaloid.

New Amphibian Alkaloids from Australian Frogs. A tricyclic tryptamine monoterpene alkaloid has been discovered in skins of the burrowing frog Pseudophryne coriacea. Two presumptive decomposition products were also detected, evidently arising from methanolysis of the tricyclic tryptamine alka-

loid. The structures are reminiscent of physostigmine. In addition, extracts from this Australian frog contain isomers of pumiliotoxin B and related alkaloids, one of which is many fold more potent in cardiac and other systems than pumiliotoxin B.

Potent Ca^{++}-ATPase Inhibitors as Environmental Contaminants of Alkaloids. Purified samples of the amphibian alkaloid pumiliotoxin B were found to contain trace impurities that manifested the inhibitory activity toward two ATPases originally ascribed to the alkaloid. One of these inhibitors, identified as bis-(2-hydroxy-3-tert-butyl-5-methylphenyl)methane(bis-phenol) is the most potent inhibitor known for the Ca^{2+}-dependent ATPase of skeletal muscle sarcoplasmic reticulum. Another, 4-nonylphenol, is the most potent inhibitor of calcium uptake into storage sites in cardiac sarcoplasmic reticulum. The antioxidant butylated hydroxytoluene was also detected in alkaloid preparations. Such phenolic compounds are widely used in plastics and rubber formulations, including rubber-lined caps of various vials. Photooxidation products were also detected and the action of pyrex-filtered visible light on such phenols have been ascertained as well as their reactions with the halogen-containing oxidants iodine, bromine, n-bromosuccinimide and t-butylhypochlorite. A variety of products, including various quinones, dimers and methanol adducts have been identified.

Histrionicotoxins as Antagonists of Ion Channel Ligands. A series of eight histrionicotoxins and two synthetic analogs inhibit binding of [^{3}H]-batrachotoxinin B to sites on voltage dependent sodium channels in brain membranes. Perhydrohistrionicotoxin (IC_{50} 0.33 μM) and octahydrohistrionicotoxin (IC_{50} 1.2 μM) are comparable in activities to potent local anesthetics. Histrionicotoxin (IC_{50} 17 μM) and the other histrionicotoxins and much less potent. The histrionicotoxins also inhbit binding of [^{3}H]phencyclidine to putative potassium channels in brain membranes. Histrionicotoxin (IC_{50} 15 μM) and the other histrionicotoxins are much more potent than perhydrohistrionicotoxin (IC_{50} 200 μM), but are at least 200-fold less potent than phencyclidine. The histrionicotoxins enhance binding of [^{3}H]nitrendipine to sites on calcium channels in brain membranes, with the exception of perhydrohistrionicotoxin, which inhibits binding. Structure activity relationships at these channel sites and at the sites for noncompetitive blockers on the nicotinic acetylcholine receptor channel (AChR) complex differ. The histrionicotoxins and more potent at the sites on the AChR complex than at sites on other channels with the exception of perhydrohistrionicotoxin, which has comparable potency at the AChR complex and sodium channels.

Gephyrotoxins and Indolizidines as Noncompetitive Blockers of the Acetylcholine Receptor Channel Complex. The interactions of eighteen natural and synthetic gephyrotoxin and indolizidine alkaloids with binding sites on nicotinic actylcholine receptor channel complex from Torpedo californica electric organ were investigated using two radiolabelled probes, [^{3}H]perhydrohistrionicotoxin and [^{3}H]phencyclidine. Both gephrotoxins and indolizidines were moderately active inhibitors of the binding of these probes (K_i's = 0.1-20 μM), but did not interact with the acetylcholine binding site. Structure-activity relationships indicate an important contribution of hydro-

phobic interactions to both gephyrotoxin and indolizidine binding. The stereoconfiguration of the alkaloids had little effect on binding. Carbamylcholine enhanced the affinity of certain alkaloids up to 6 to 8-fold suggesting that interactions with open or desensitized conformations of the AChR complex are favored over interactions with resting conformations.

Binding Sites for a Radioactive Batrachotoxin Analog. Batrachotoxin-A [^{3}H]benzoate ([^{3}H]BTX-B) binds specifically and with high affinity (K_D 48 nM) to sites (B_{max} 2.1 pmol/mg protein) associated with voltage-dependent sodium channels in rodent brain vesicular preparations. High affinity binding requires the presence of scorpion (Leiurus) venom and a membrane potential. Local anesthetics antagonize the binding. Nonspecific binding is defined in the presence of veratridine. In particulate preparations from electroplax of the eel Electrophorus electricus, [^{3}H]BTX-B binds with a K_D of about 140 nM and a B_{max} of 2.5 pmol/mg protein in the presence of scorpion venom. Higher concentrations of scorpion venom are required to enhance binding in Electrophorus preparations than in brain preparations. Local anesthetics antagonize binding in Electrophorus preparations with potencies similar to those in brain preparations. Veratridine and batrachotoxin (BTX) are less potent in blocking binding in Electrophorus than in brain preparations. It appears likely that binding in Electrophorus preparations is primarily to membrane fragments rather than vesicular entities as in brain. Binding of [^{3}H]BTX-B to particulate preparations from electroplax of the ray Torpedo californica and the catfish Malapterurus electricus is mainly nonspecific. Scorpion venom does not enhance total binding and local anesthetics are not effective in antagonizing binding.

Effect of Local Anesthetics on the Dissociation Rate of [^{3}H]BTX-B from the BTX-Binding Site on Sodium Channels. Local anesthetics increase the dissociation rate of [^{3}H]BTX-B from the BTX-binding site in the presence of excess competitive BTX ligands such as BTX, BTX-B, aconitine, and veratridine. This enhancement of the dissociation rate was observed with 15 classical local anesthetics ranging in potency from dibucaine (Ki = 0.6 μM) to procaine (K_i = 50 μM). The concentration dependence of this effect was determined from the increase in rate at several anesthetic concentrations. The values (ED 50) for local anesthetics in the presence of a competitive ligand yield a 1:1 correlation (R = 0.96) with the K_i values for inhibition of [^{3}H]BTX-B binding. This result indicates that local anesthetics reduce the binding of BTX by allosterically reducing the affinity of the binding site for BTX rather than competing with BTX binding per se. In the absence of excess competitive ligands, local anesthetics still induce a linear (when expressed as log 10 of % control), concentration dependent increase in the dissociation rate of [^{3}H]BTX-B, although at higher concentrations than in the presence of a competitive ligand. The ED50 values for local anesthetics alone showed little if any correlation with the K_i values for the inhibition of binding of [^{3}H]BTX-B, suggesting that that binding site(s) for local anesthetics is a separate entity from the binding site for BTX. The concentration dependence of the local anesthetic effect clearly distinguishes between the effect of a true competitive ligand: Increasing concentration of true competitive ligands fail to increase the dissociation rate beyond the equi-

librium value. The dissociation constant of [^{3}H]BTX-B from sodium channels in guinea pig cerebral cortical synaptoneurosomes is 0.009/min (half time = 31 min) as determined in the presence of BTX, BTX-B, aconitine and veratridine. Employing this criteria for true competitive ligands we have demonstrated that besides the various toxins, reserpine appears to behave like a true competitive ligand.

Pumiliotoxin B as a Cardiotonic Agent. The cardiotonic activity of pumiliotoxin B in guinea pig atria is markedly dependent on the 6-alkylidene side chain. Pumiliotoxin A, which differs only in lacking the 16-hydroxy moiety is much less active than pumiliotoxin B. Alterations in the configuration of the 15- and/or 16-hydroxy moieties in synthetic isomers of pumiliotoxin B reduces cardiotonic activity, while lack of such moieties or replacement with methoxy or ketone moieties yields cardiodepressant compounds. The cardiotonic alkaloids of this class stimulate phosphatidyl inositol turnover in atrial and brain preparations and sodium influx in brain preparations as do a variety of other cardiotonic agents including ouabain and pyrethroids. It appears that activation of sodium flux and a resultant stimulation of phosphatidyl inositol breakdown plays a role in the cardiotonic activity of pumiliotoxins and perhaps a variety of other cardiac stimulants. Phorbolesters that mimic diacylglyceride activation of protein kinase C have cardiotonic activity suggesting that both calcium mobilization by inositol phosphates and activation of protein kinase C by diacylglycerides may be important to activities of pumiliotoxins.

Pumiliotoxin B as a Myotonic Agent. Pumiliotoxin B not only increases the electrical excitability of motor nerve terminals, but also evokes repetitive potentials in response to a single stimulus. It is concluded that pumiliotoxin B acts both on the nerve terminal and muscle fibers to produce repetitive activity by altering the sodium conductance inactivation. Subsequent effects on calcium influx and intracellular mobilization of calcium could account for the observed potentiation and prolongation of muscle twitch in striated neuromuscular preparations.

SECTION ON PHARMACODYNAMICS

Rigid and Semirigid Agonists for Autonomic and Central Nervous System Receptors

Isoarecolone methiodide (ISO) is one of the most potent nicotinic agonists known when evaluated at the frog neuromuscular junction and in the displacement of [^{3}H]nicotine binding in rat brain membranes. 3,4-Dihydro-ISO and 2,2-dimethyl-5-ketodecahydroisoquinolinium iodide are less active. Geometric properties of these substrates using energy minimized conformations have been determined using molecular modeling techniques. 2-Phenylbenzoates of 3-(diethylamino)propanol, (diethylamino)ethanol and 1-methyl-4-piperidinol are as potent, or slightly more potent, as phenytoin in the MES assay. These anticonvulsants, as well as phenytoin, antagonize the binding of the batrachotoxin analogue to sodium channel sites. Certain structural similarities are evident between the 2-phenylbenzoates and phenytoin.

SECTION ON PHARMACODYNAMICS

Pharmacodynamics Amines and Enzymes Involved in their Metabolism

The presence of COMT in separately cultured cerebromicrovascular endothelial and smooth muscle cells. The activity of COMT was investigated in cultured and propagated cerebromicrovascular endothelial and smooth muscle cells using HPLC and immunocytochemistry. The presence of COMT was demonstrated in both cell types. Endothelial cell cultures contained approximately 2-3 times as much COMT as present in smooth muscle cultures. The demonstration of COMT in the smooth muscle cells in addition to the endothelium indicates that the enzymatic barrier to catechols is not limited to capillaries. The primary constitutents of the blood-brain barrier, the presence of COMT in both cell types of the cerebromicrovasculature by providing a mechanism for the inactivation of catechols may be an important mechanism by which neurohormonal regulation of the blood-brain barrier, cerebral blood flow and blood pressure can be maintained under normal conditions. This protective mechanism might be deficient in cerebrovascular disease. Similar studies with cell cultures derived from spontaneously hypertensive and stroke-prone rat strains are in progress.

Localization of COMT in rat oviduct and macrophages of corpus lutea of rat ovary. Immunocytochemical methods utilizing a COMT-specific antisera were used to extend our previous study of the localization of COMT in the reproductive system of the rat. COMT-specific immunoreactive deposits were found in the cytoplasm of macrophages in the corpus lutea of rat ovary, epithelial cells of the oviduct and the glandular epithelial cells of the nonpregnant uterus. The pattern of localization observed in the extraneuronal elements suggested that COMT may function in the extraneuronal inactivation of catechols in the ovary, oviduct, and uterus. Studies are in progress using monoclonal antibodies specific for rat macrophages in conjunction with antisera for COMT to determine if all macrophages contain COMT or if there is a subpopulation of COMT specific-macrophages. Preliminary results in rat lymph node suggest that there is a subpopulation of macrophages which contain COMT.

Localization of COMT in delayed implantation, pseudopregnant, and postpartum rat uteri. In nonpregnant rats, the COMT positive immunocytochemical reaction product was only observed in the cytoplasm of glandular epithelial cells during estrus and diestrus and early pregnancy. On day 3 and 4 of pregnancy the luminal epithelial cells became COMT positive. An identical pattern of glandular COMT and the same time dependent appearance of COMT in the luminal epithelium was observed in pseudopregnancy. In delayed implantation animals the appearance of COMT in the luminal epithelium was delayed until day 4-5. Ovariectomy on day 0 or day 1 of pregnancy prevented the normal appearance of COMT in the luminal epithelium. Progesterone replacement at ovariectomy resulted in the normal appearance of COMT. Ovariectomy on day 2 or 3 of pregnancy had no effect on the normal appearance of COMT nor did progesterone treatment. There appeared to be two types of localization of

COMT in the luminal epithelium: 1) antimesometrial, which appears to be related to the implantation phenomena, and 2) a general, uniform increase in COMT related to an increased progesterone level. The study is being continued to more precisely determine the factors which account for the rather sudden appearance of COMT in the luminal epithelium of rat uteri and to determine the hormonal or implantation-related components which lead to the two types of COMT distribution.

Localization of COMT in the vascular system. Studies attempting to resolve a controversy over the site of COMT localization in vascular systems have been continued. Our earlier demonstration of COMT in the endothelium of rat aorta by immunocytochemical methods has resulted in an effort to confirm this finding by biochemical measurements and to extend the study to other species and other sites in the vascular tree (see section a. above). Methods for the microassay of COMT activity by HPLC have been developed using both catechol steroids and catecholamines as the catechol substrate. The O-methylation of 2-hydroxyestradiol in segments of rabbit thoracic aorta were found to be significantly less than the activity in segments of abdominal aorta. The site of O-methylation appears to be predominantly associated with endothelial cells from the intimal surface of these aortal segments. Immunochemical examination of these segments of rabbit aorta are in progress.

Effect of ring-fluorination on the adrenergic agonist properties of phenylephrine. 2-F, 4-F, and 6-fluorophenylephrine (FPEs) were synthesized from the corresponding 3-hydroxybenzaldehydes. As with the norepinephrine and isoproterenol analogs, the adrenergic agonist properties of the fluorophenylephrines were altered compared to those of phenylephrine. The order of affinities of these analogs for alpha 1-adrenergic receptors as determined by the specific displacement of radioligands from alpha 1-receptors on brain membranes was the same as the order of potencies for three alpha-1 adrenergic agonist systems (guinea-pig aortic strip, stimulation of phosphatidylinositol turnover and potentiation of 2-chloroadenosine-induced accumulation of cyclic AMP in guinea pig cerebral cortical synaptoneurosomes). The order of potency was 6-FPE > PE > 4-FPE > 2-FPE. The order of affinities for alpha-2 adrenergic receptors as determined by the specific displacement of [H3]clonidine binding the brain membranes was 6-FPE > PE > 4-FPE = 2-FPE. In contrast, the order of potencies for inhibition of forskolin-stimulated adenylate cyclase activity in human platelet membranes via an alpha-2-adrenergic receptor was PE = 6-FPE > 4-FPE >> 2-FPE. The FPEs and PE were partial agonists compared to epinephrine in human platelets. The affinities of these compounds for beta-adrenergic receptors as determined by displacement of the specific binding of [^{3}H]dihydroalprenolol to brain membranes are 2-FPE > PE > 4-FPE >> 6-FPE. The FPEs and PE exhibited positive chronotropic and inotropic effects in the isolated guinea pig atria ostensibly through activation of a beta-adrenergic receptor, since pindolol blocked the response while prazocin did not. 6-FPE appeared less active than the other FPEs and PE in the atria. In fat cell membranes, 2-FPE was more potent than PE in stimulating adenylate cyclase via a beta-adrenergic receptor, while 4-FPE and 6-FPE were inactive. Both 2-FPE and PE were partial agonists in fat cells compared to isoproterenol. Of the three FPEs, 6-FPE represents a more potent and more selec-

tive agonist for alpha-adrenergic receptors than PE. While 4-FPE and, in particular, 2-FPE are less potent and selective as alpha-adrenergic agonists. A rationale for the observed fluorine-induced alteration in the potency and selectivity of the FPEs for alpha- and beta-adrenergic systems was proposed based upon fluorine-induced conformations due to electrostatic repulsion of fluorine and the benzyl hydroxyl group.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 31100-21 LBC
formerly
Z01 AM 31100-20 LBC

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pharmacologically Active Compounds from Amphibians and Other Natural Sources

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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SECTION

Section on Pharmacodynamics

INSTITUTE AND LOCATION

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	3.5	1.0

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☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Over two hundred pharmacologically active alkaloids have been identified in extracts of frog skins. Most of these represent unique structures and serve the frogs in chemical defense against predators. New structural classes of amphibian alkaloids include tricyclic tryptamine monoterpenes, 4-hydroxy 2,6-dialkylpiperidines, and non-hydroxylated congeners, 2,5-dialkylpyrrolidines, 3,8 dialkylindolizidines and amidines. The alkaloids exhibit a range of pharmacological activities. The steroidal batrachotoxins are potent activators of voltage-dependent sodium channels. A radioactive batrachotoxin analog binds to sites associated with sodium channels in brain and electroplax and provides a unique tool for probing mechanisms, both allosteric and direct through which local anesthetics and other drugs antagonize sodium channel opening. The spiropiperidine historionicotoxins, the tricyclic gephyrotoxins and the indolizidine alkaloids are noncompetitive allosteric blockers of nicotinic acetylcholine receptor channel complexes. In addition, histrionicotoxins interact with phencyclidine-sensitive sites on central potassium channels, and affect binding of dihydropyridines to sites on central calcium channels. Pumiliotoxin B, an alkylidene-hydroxyindolizidine, is a potent cardiotionic and myotonic agent, whose action and that of certain other cardiac stimulants appears to involve activation of sodium flux and a resultant stimulation of phosphatidyl inositol breakdown leading to both calcium mobilization and activation of protein kinase C. Certain hydrophobic bis- and monophenols detected as environmental contaminants of alkaloid fractions are very potent inhibitors of Ca^{++}-ATPase from muscle sarcoplasmic reticulum.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 31101-18 LBC
formerly
Z01 AM 31101-17 LBC

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Pharmacodynamics Amines and Enzymes Involved in their Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	C. R. Creveling	Research Chemist	LBC, NIDDK
Others:	J. W. Daly	Chief	LBC, NIDDK
	G. A. Lewandowski	Guest Worker	LBC, NIDDK

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Section on Pharmacodynamics

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.9	0.6	0.3

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The chemistry, biochemistry, physiology, and pharmacology of biogenic amines, their amino acid precursors and metabolic products, and various synthetic derivatives thereof have been investigated. The areas of specific interest are: 1) Determination of the primary sequence of catechol-O-methyltransferase (COMT) and construction of a COMT-specific cDNA probe; 2) The immunohistochemical localization of COMT in malignant, physiologically and hormonally modified, and normal tissues from rodent, and human at the light and electron microscopic level including: examination of the temporal and hormonal relationship between uterine epithelial COMT during the course of pregnancy in rat and hamster, study of the relationship between breast ductal epithelial-COMT and lactation in rat and mouse, study of COMT in both normal and malignant breast tissue in women with regard to the relationship between both type and extent of malignancy, the occurrence of increased COMT levels in gynomasty in man, and the distribution of COMT in endothelial and smooth muscle cells in the vascular system; 3) A study of the chemical and biological properties of various fluoro derivatives of biogenic amines, amino acids, and related compounds including studies of: the electrochemical, redox properties and electron densities of fluorocatechols, the interaction of fluorophenylephrines and fluoroepinephrines with both alpha and beta receptor systems, interaction at the active site of COMT and monoamine oxidase A and B, the uptake and metabolism of 6-fluorodopa and 6-fluorodihydroxyphenylserine in vitro and in vivo, the application of fluorine-18 analogs of dopa on dihydroxyphenylserine as PETT scanning agents for dopamine and norepinephrine neurons in the intact animal; the mechanism of toxicity of 2-fluorohistidine in leukemic mice, and the uptake and mechanism of toxicity of 6-fluorodopa in cultured pheochromocytoma and melanoma cell lines.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 31102-15 LBC
formerly
Z01 AM 31102-14 LBC

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cyclic Nucleotides and Other Second Messengers in the Nervous System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.0	3.5	2.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Adenosine modulates a variety of physiological functions through interactions with A_1 and A_2 adenosine receptors, where agonists can mediate inhibition and stimulation, respectively, of adenylate cyclase. Adenosine analogs, in particular the N^6-substituted compounds, are more potent at A_1 receptors than at A_2 receptors. The subregion of the adenosine receptor that interacts with the N^6-substituent is different for A_1 and A_2 receptors, particularly with respect to phenyl interactions, bulk tolerance and stereoselectivity. Xanthines are classical antagonists for adenosine receptors and many of their pharmacological actions may be due to blockade of adenosine receptors. Caffeine and theophylline are virtually non-selective for A_1 and A_2 receptors. Replacement of the methyl groups of theophylline with n-propyl or larger alkyl groups yields xanthines with selectivity for A_1 receptors, particularly when combined with an 8-phenyl moiety. Most 1,3-dialkyl-8-phenylxanthines are very water insoluble, but incorporation of polar aryl substituents, such as para-sulfo or para-carboxy to increase solubility, results in marked reduction in potency and selectivity. Certain analogs of caffeine in which the methyl group at the 1- or 7-position is replaced with a propargyl or propyl group display selectivity for A_2 receptors. The profile of a series of adenosine analogs or of xanthine antagonists can be used to define the class of adenosine receptors. Receptor agonists and agents that enhance influx of sodium into brain synaptoneurosomes enhance turnover of phosphatidylinositols resulting in accumulations of inositol phosphates. Such receptor agonists and sodium agents concomittantly augment accumulations of cyclic AMP elicited by forskolin and by A_2-adenosine receptors. Phorbol esters, which like the diacylglycerides formed during phosphatidylinositol metabolism, activate protein kinase, also augment accumulations of cyclic AMP both in brain tissue and in pheochromocytoma cells.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 31103-09 LBC
formerly
Z01 AM 31103-08 LBC

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Receptors for Neurotransmitters and Drugs in Brain and Peripheral Tissues

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.5	5	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

High affinity, stereospecific recognition sites (receptors) for neurotransmitters, neuromodulators, and many clinically useful drugs have been identified in both peripheral tissues and the central nervous system. The interaction of a neurotransmitter, neuromodulator or drug with a specific recognition site initiates a series of events (for example, the opening of an ion channel or activation of an enzyme) resulting in either a physiological/behavioral response (in the case of a neurotransmitter or neuromodulator) or a pharmacological effect (in the case of a drug). Furthermore, such observations suggest that endogenous substances may also be present that mimic (or antagonize) the effects of exogenously applied substances. Studies are in progress to characterize "recognition-effector" systems, and to link novel recognition sites to effector systems in both peripheral tissues and the central nervous system in order to define the physiological roles of these systems. "Recognition-effector" systems under study include: a) the benzodiazepine/GABA receptor chloride ionophore complex; b) the glycine-gated chloride ionophore; c) "peripheral-type benzodiazepine receptors (in both peripheral tissues and the central nervous system); d) receptors for central stimulants (e.g. amphetamine, methylphenidate); e) recognition sites for hallucinogens (phencyclidine), and f) recognition sites for compounds that regulate voltage-sensitive calcium channels.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 31104-18 LBC
formerly
Z01 AM 31104-17 LBC

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Enzymatic Oxidation of Drugs to Toxic and Carcinogenic Metabolites

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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	L. Pannell	Expert	LBC, NIDDK
	S. G. Grossman	Staff Fellow	LBC, NIDDK
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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
10.5	9.5	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The primary goal has been the elucidation of the structures of reactive metabolites which are responsible for the carcinogenic, cytotoxic, and mutagenic activity of benzo[a]pyrene and other polycyclic aromatic hydrocarbons. The approach taken consists of: i) synthesis of primary oxidative metabolites as well as selected secondary oxidative metabolites, ii) study of the metabolism of these hydrocarbons with liver microsomes, as well as with purified and reconstituted cytochrome P-450 systems with and without epoxide hydrolase, iii) tests for inherent mutagenicity of the synthetic metabolites toward bacterial and mammalian cells, iv) elucidation of the roles of the cytochrome P-450 system and epoxide hydrolase in potentiating or obliterating the mutagenicity of these metabolites, v) determination of the carcinogenic activity of these compounds, vi) determination of the rate of formation and nature of the products formed when reactive metabolites such as arene oxides and diol epoxides react with biopolymers and less complex model compounds, and vii) search for compounds capable of preventing the tumorigenic action of bay-region diol epoxides. Current chemical studies have included resolution and assignment of absolute configuration to benzo[c]-phenanthrene 5,6-oxide as well as the K-region trans dihydrodiols of nine polycyclic hydrocarbons. A novel method for complete ^{1}H and ^{13}C NMR assignment of complex hydrocarbons has been developed. Metabolism studies have shown that benzo[c]phenanthrene may be a weak carcinogen due to poor conversion to bay-region diol epoxides and that 7,12-dimethylbenz[a]anthracene is metabolized to its (5S,6R)-oxide in accord with theoretical predictions. Tumor studies on optically active, bay-region diol epoxides of benzo[c]phenanthrene have identified the (4R,3S)-diol-(2S,1R)-epoxide as the most potent carcinogenic metabolite of a hydrocarbon yet described. A new liver microsomal epoxide hydrolase has been characterized and shown to be selectively inactivated by a mechanism based inhibitor.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 31105-01 LBC

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Rigid and Semirigid Agonists for Autonomic and Central Nervous System Receptors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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COOPERATING UNITS *(if any)*

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	1.5	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Isoarecolone methiodide is one of the most potent nicotinic agonists known at the frog neuromuscular junction and in inhibiting [^{3}H] nicotine binding in rat brain membranes. 3,4-Dihydroisoarecolone methiodide and 2,2-dimethyl-5-keto-decahydroisoquinolinium iodide, though structurally similar, were less active. Minimum energy conformations of these ligands were calculated by molecular modeling. 2-Phenylbenzoates of open-chain aminoalcohols and 1-methyl-4-piperidinol had anticonvulsant activities comparable to that of phenytoin in a maximal electroshock seizure assay. Models show certain structural similarities between these esters and phenytoin. Both classes antagonize the binding of a batrachotoxin analogue to sodium channel sites.

Annual Report of the Laboratory of Molecular Biology

National Institute of Diabetes and Digestive and Kidney Diseases

The Laboratory of Molecular Biology has as its principal goal the understanding of biological processes at the molecular level. The research program involves application of both theoretical and experimental methods to a wide variety of problems in molecular genetics, regulation of gene expression in eukaryotes, mechanisms of DNA replication, nucleic acid and protein structure, bioenergetics and transport properties. Specific areas under investigation include the structures and chemical properties of biologically important materials. These involve studies of the organization of DNA and proteins within the eukaryotic nucleus, investigations of enzyme and immunoglobulin structures by X-ray diffraction, investigation of polynucleotide chemistry and structure by synthetic and spectroscopic methods, and of protein interactions by calorimetric methods, studies of the conformations of supercoiled DNA and their effect on biological properties, and theoretical studies of the mechanism of energy conversion in biology, muscle contraction, microtubule formation, ion transport and biochemical kinetics. Other investigations are concerned directly with biological processes. These include studies of the process of transformation by the tumor virus SV40, of immunoglobulin gene rearrangement, of nonheritable antibiotic resistance, of the effects of macromolecular crowding, and of the mechanisms of genetic recombination and DNA replication. Important advances have been made in these areas this year.

Chromatin Structure and Function

We have continued our studies of chromatin structure, in order to learn how DNA is packaged within eukaryotic nuclei, and in particular to learn how the structure is altered in the neighborhood of genes that are being expressed. We have studied the way in which chromatin containing expressed genes folds to form compact fibers, and we have shown that at high salt concentrations such chromatin is not capable of the full compaction that occurs in inactive chromatin. We have also continued our studies of the structure of chromatin in the neighborhood of the chicken adult β globin gene, isolated from the nuclei of erythrocytes in which the gene is expressed. We had earlier identified sites within the nuclease hypersensitive domain in the 5' flanking region of the active gene that are binding sites for DNA sequence-specific proteins which we had partially purified. We have now shown that there are at least three different proteins involved, each of which binds to a distinct portion of the 5' flanking region, and we have studied their appearance in red cells as a function of developmental stage. A similar analysis has been undertaken for one of the α globin genes. In order to determine the biological function of these and other globin DNA sequences, we have developed a method for transfecting DNA into primary chicken erythrocytes at various stages of development. The method makes use of controlled, specific red cell lysis to obtain high levels of expression of transfected DNA. The method has led to the detection of a new regulatory region with the properties of an enhancer in the 3' flanking region of the β globin gene. The region with enhancer activity is the site of another hypersensitive domain we had previously identified. We have identified specific protein factors that bind to the region, and we have used footprinting methods to determine the binding sites precisely.

Enzyme Structure

The three-dimensional structure of the aspartyl protease from Rhizopus chinensis has been refined with the high resolution X-ray data measured last year. The final R-factor is 14.3% for the 30,000 data and the model has an RMS departure from ideality of 0.01Å. Some 370 water molecules have been located on the surface of the enzyme. One of these, positioned between the two active aspartyl groups is believed to be the water necessary for catalytic attack on the carbonyl carbon of the peptide bond. Preliminary examination of several inhibitors of the enzyme has been made and these are now being refined with a view of providing more insight into the mechanism of action. One of these inhibitors is also a potent inhibitor of human renin and a study of its binding to the fungal enzyme may suggest ways in which it can be made increasingly effective against renin.

The X-ray analysis of tryptophan synthase from Salmonella typhimurium has been continued. A number of X-ray data sets have been measured and are being analyzed to provide data for structure determination.

Three-Dimensional Structure of Proteins of the Immune System

Further studies of the crystal structures of antibodies with specific binding properties have been carried out. These include two antilysozyme monoclonal antibodies and one antigalactan.

The X-ray data measured last year for the Fab lysozyme complex of a monoclonal antibody to lysozyme (Hy10) has been extensively analyzed by the methods of isomorphous replacement and molecular replacement. Data have been measured for two crystal forms of another complex (Hy5) and are also being analyzed by molecular replacement.

The three-dimensional structure of an antigalactan Fab (JS39) has been refined to 2.7Å and further X-ray data have now been measured that will permit the analysis to be extended to 2.0Å.

Comparison of Structural and Energetic Effects in Ribo- and Deoxypolynucleotides. Poly 2-Amino-8-methyldeoxyadenylic Acid

A bulky methyl group placed in the 8-position of poly A shifts the glycosidic equilibrium toward the syn conformation and, though it is far removed from the Watson-Crick binding sites, completely prevents base pairing in the ribopolynucleotide series. Substitution of a 2-NH_2 group in adenine permits three hydrogen bonds to U or T and reverses the inhibition of pairing caused by the 8-Me group. CD spectra show that the homopolymer undergoes a major conformational change on base pair formation, adopting in the helix a conformation like those of other 2-NH_2A complexes. Evidently the third hydrogen bond supplies the free energy to drive the conformation to the anti form, though the 8-Me group is strongly destabilizing. Recent results with the corresponding deoxy polymer are in marked contrast. Heteroduplex formation occurs readily, and the destabilizing effect of the 8-Me group is relatively small: a depression of the transition temperature by 7° to 30° rather than the 70° or more observed in the ribo series. The greater flexibility of the deoxypolynucleotide chain and smaller size of the 2'-CH_2 compared to the 2'-CHOH evidently reduce the steric effects of bulky 8-substituents and markedly change the properties of the polymer.

Macromolecular Assembly Processes. The complex thermal denaturation profile of the bacteriophage T4 head particle in the small unexpanded form has been identified. The increase in thermal stability arising in the transformation to the expanded lattice form producing large particles has been described.

Influences of Macromolecular Crowding on Biochemical Systems

T4 polynucleotide kinase rapidly loses activity during its reaction on duplex DNA termini. Addition of high concentrations of nonspecific polymers reverses or prevents this inactivation. In contrast, additions of related materials of lower molecular weight are relatively ineffective in stabilizing the kinase. Such a pattern suggests that the stabilizing effects of polymers on kinase activity are due to macromolecular crowding. An effect of crowding on the known tendency of the kinase to undergo oligomerization reactions is consistent with our observations. This effect of polymers on the kinase can be exploited to greatly increase the amount of reaction obtainable on 5'-hydroxyl groups of duplex DNA substrates located at recessed or blunt ends or at "nicks" which are otherwise difficult to effectively phosphorylate or dephosphorylate.

Macromolecular crowding was found to cause no stiking change in the processivity of the T4 DNA ligase under several reaction conditions.

The Patent Office has approved an application on behalf of the Government, and we await the issuance of a patent based on our previous studies of polymer-stimulated ligation of DNA.

Mammalian Origin of DNA Replication

We have isolated a number of cloned monkey DNA fragments, some of which are believed to contain origins of DNA replication. Two of the fragments contain a class of moderately reiterated highly dispersed sequences known as the "O-family". We have been attempting to isolate a sequence-specific binding protein to these fragments.

Another of the fragments contains the consensus sequence thought to be necessary for providing origin function for replication in yeast (the autonomously replicating "ars" sequence). The fragment has been cloned in yeast tester plasmids and does appear to have very weak ars function.

We have sequenced a large portion of monkey mitochondrial DNA and compared it with the human sequence.

We have prepared monoclonal antibody to DNA molecules containing cruciform structures. We have shown that the monoclonal antibody protects against the action of Endo VII but not single-stranded nucleases, suggesting that the antibody recognizes the base of the cruciform structure.

Noninheritable Antibiotic Resistance

Our previous work showed that certain weak acids, especially aspirin and salicylate, induce nonheritable resistance to ampicillin, chloramphenicol, nalidixic acid and tetracycline in Escherichia coli. Similar results have now been obtained

in various other bacteria including certain Bacillus, Klebsiella, Salmonella, Shigella and Vibrio but not Streptococcus or Staphylococcus species. Futhermore, acetaminophen (Tylenol) has also been found to induce drug resistance.

Novel Recombination Systems

The location of the pgl (phosphogluconolactonase) gene of Escherichia coli on a 5.8 kb Kpn I fragment has been determined by insertional mutagenesis. The gene encodes a 42 KD protein, as observed in maxicell preparations. sapA, a gene involved in transpositional activation of the cryptic bgl operon, appears to be identical to pgl since mutations that inactivate one function inactivate the other and result in the loss of expression of the 42 KD protein.

Replication of ColE1 DNA

Studies on the mechanism of ColE1 DNA replication and its regulation have been continued. A nascent transcript (RNA II) by RNA polymerase that starts 555 nucleotides upstream of the replication origin forms a persistent hybrid with the template DNA near the origin. The hybridized transcript is cleaved by RNase H and used as the primer of DNA synthesis by DNA polymerase I. Primer formation is affected by point mutations. Each of them affects a specific stage of primer formation and inactivated primer. Studies on RNA II structure by a computer program under constraints which are based on biochemical and genetic data show that functional RNA II has a unique secondary structure that folds in a specific tertiary conformation.

Primer formation is regulated by a plasmid-specified small RNA (RNA I). Synthesis of RNA I starts 445 base pairs upstream of the replication origin, proceeds in the direction opposite to that of RNA II synthesis, and terminates near the initiation site of RNA II synthesis. Thus this RNA is an anti-sense RNA with respect to RNA II. RNA I binds to RNA II at the complementary region. This binding results in inhibition of formation of the secondary structure necessary for primer formation. These works firmly confirm our previous discovery of biological regulation by an anti-sense RNA. Kinetic analysis of binding of RNA I to RNA II revealed that the binding starts by reversible interaction between loops of folded structures of these RNAs. This interaction facilitates stable binding that starts at the 5'-end of RNA I and propagates step-wise to its 3'-end.

Primer formation is also regulated by a 63-amino acid protein specified by the plasmid. The protein stimulates binding of RNA I to RNA II by affecting the reversible interaction of these RNAs. This enhances the inhibitory action of RNA I. Inhibition of primer formation by RNA I in the presence or absence of the protein determines the copy number of a plasmid in a cell and the incompatibility between related plasmids.

Energy Conversion in Biology

A large number of different topics have been studied in the general field of free-energy transduction. The most important areas in which progress has been made are the dynamics of actin and mictotubule assembly and disassembly, the potential role of nonstationary electric fields in biological free-energy transduction, the theory of the control of microbial growth, the spatial profile of electric potentials across biological membranes, the assay methods for channelled metabolism, and the role of protons in chemiosmotic systems.

Statistical Thermodynamics of Protein and Polynucleotide Systems

A theory has been developed for the calculation of polymer-activated enzymatic activities based on the one-dimensional piggy-back binding model previously studied. It was found that by invoking differential cooperativities between gyrase molecules and between gyrase and ATP-bound gyrase molecules, the theory is able to explain the observed differences in the ATP hydrolysis rates by DNA gyrase in the presence of λ DNA and short DNA fragments.

Studies of Immunoglobulin Gene Rearrangement

Plasmids were designed and constructed to allow a sensitive and specific measure of immunoglobulin V-J gene recombination in immature lymphocytes. Recombination at the specific sites leads to either the deletion or the inversing of a segment of DNA, depending on the arrangement of V and J recognition sequences in the plasmid. In both cases, recombination leads to the expression of a chloramphenicol-resistance gene, which is readily measured by transforming the recombined DNA into bacterial cells. With this assay, a number of pre-B lymphocyte cell lines have been shown to produce recombinants.

Studies of Complexes Between DNA Gyrase and DNA

Complexes of DNA gyrase with defined DNA fragments from 127 to 256 base pairs in length have been studied by the method of electrodichroism. The results suggest that DNA is wrapped around the enzyme in a single loop of 110 base pairs, with the entry and exit points close together. The rest of the DNA extends outward in two tails with an angle of about 120° between them. In the presence of ATP or a non-hydrolyzable ATP analog, the tails are wrapped back and bound to the enzyme core. This process is considered to represent an intermediate stage in the supercoiling reaction of DNA gyrase.

The complexes are also being studied by neutron scattering methods. Preliminary results are consistent with the model of a single turn of DNA bound to the enzyme.

Studies on the Mechanism of Genetic Recombination

The major objective of this project is to uncover the enzymatic steps involved in various genetic rearrangement reactions and to study the mechanism of action of the enzymes involved. We are currently concentrating our efforts on the mechanism of the transposition-replication reaction of bacteriophage Mu. Recent developments include the establishment of an *in vitro* reaction system for the study of replicative transposition of bacteriophage Mu. The *in vitro* reaction yields cointegrate products as well as simple insertion products and requires both the A and B gene products of phage Mu along with other bacterial proteins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 33000-20 LMB
Formerly
Z01 AM 33000-19 LMB

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of Functions Involved in Genetic Recombination

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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COOPERATING UNITS *(if any)*

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Dr. A. Wlodawer, National Bureau of Standards, Gaithersburg, MD
Ms. S. Krueger, University of Maryland, College Park, MD

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Section on Metabolic Enzymes

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1.5	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Complexes of DNA gyrase with defined DNA fragments from 127 to 256 base pairs in length have been studied by the method of electro-dichroism. The results suggest that DNA is wrapped around the enzyme in a single loop of 110 base pairs, with the entry and exit points close together. The rest of the DNA extends outward in two tails with an angle of about 120° between them. In the presence of ATP or a non-hydrolyzable ATP analog, the tails are wrapped back and bound to the enzyme core. This process is considered to represent an intermediate stage in the supercoiling reaction of DNA gyrase.

The complexes are also being studied by neutron scattering methods. Preliminary results are consistent with the model of a single turn of DNA bound to the enzyme.

GPO 914-910

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 33001-02 LMB
Formerly
Z01 AM 33001-01 LMB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies of Immunoglobulin Gene Rearrangement

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Martin Gellert	Chief, Section on Metabolic Enzymes	LMB/NIDDK
	Kiyoshi Mizuuchi	Visiting Scientist	LMB/NIDDK
Others:	Joanne Hesse	Research Chemist	LMB/NIDDK
	Michael Lieber	Guest Worker	LMB/NIDDK
	Tommie McCarthy	Visiting Fellow	LMB/NIDDK

COOPERATING UNITS *(if any)*

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Laboratory of Molecular Biology

SECTION
Section on Metabolic Enzymes

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1.5	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Plasmids were designed and constructed to allow a sensitive and specific measure of immunoglobulin V-J gene recombination in immature lymphocytes. Recombination at the specific sites leads to either the deletion or the inversion of a segment of DNA, depending on the arrangement of V and J recognition sequences in the plasmid. In both cases, recombination leads to the expression of a chloramphenicol-resistance gene, which is readily measured by transforming the recombined DNA into bacterial cells. With this assay, a number of pre-B lymphocyte cell lines have been shown to produce recombinants.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 33006-08 LMB
Formerly
Z01 AM 33006-07 LMB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies on the Mechanism of Genetic Recombination

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Kiyoshi Mizuuchi,	Visiting Scientist	LMB/NIDDK
Others:	K. Adjuma	Visiting Fellow	LMB/NIDDK
	R. Craigie	Visiting Associate	LMB/NIDDK
	M. Mizuuchi	Visiting Associate	LMB/NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Section on Metabolic Enzymes

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The major objective of this project is to uncover the enzymatic steps involved in various genetic rearrangement reactions and to study the mechanism of action of the enzymes involved. We are currently concentrating our efforts on the mechanism of the transposition-replication reaction of bacteriophage Mu. Recent developments include the establishment of an in vitro reaction system for the study of replicative transposition of bacteriophage Mu. This in vitro reaction yields cointegrate products as well as simple insertion products and requires both the A and B gene products of phage Mu along with other bacterial proteins.

By making use of this cell-free reaction system, we have been able to divide the transposition reaction into two separate steps. (1) The first step involves a pair of DNA strand transfer reactions which generate an intermediate DNA molecule. The structure of this intermediate has been determined. The formation of the intermediate can be carried out by three purified protein factors; Mu A, Mu B and E. coli HU proteins. The Mu A protein binds to the Mu end DNA sequence specifically. The Mu B protein possesses an ATPase activity which is stimulated by Mu A protein and DNA. The reaction requires a transposon donor molecule which has two Mu end sequences in their proper relative orientation and is negatively supercoiled, while the transposition target DNA can be in relaxed form. Evidence was obtained which indicates that recognition of the relative orientation of two Mu end DNA sequences makes use of the energy of DNA supercoiling and requires a specific geometry of the DNA segments within the synaptic complex.

(2) intermediate DNA molecules can be converted into cointegrates by DNA replication or into simple inserts by nucleolytic cleavages and repair, in a second reaction, by an E. coli extract without Mu proteins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 34001-21 LMB
Formerly
Z01 AM 34001-20 LMB

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Chromatin Structure and Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	G. Felsenfeld	Chief, Section on Physical Chemistry	LMB, NIDDK
Others:	S.P. Clark	Visiting Fellow	LMB, NIDDK
	B.M. Emerson	Senior Staff Fellow	LMB, NIDDK
	M.M. Garner	Guest Worker	LMB, NIDDK
	H.J. Gould	Expert	LMB, NIDDK
	P.D. Jackson	Chemist	LMB, NIDDK
	B. Kemper	Guest Worker	LMB, NIDDK
	T. Kimura	Visiting Fellow	LMB, NIDDK
	C.D. Lewis	Staff Fellow	LMB, NIDDK
	J.M. Nickol	Research Chemist	LMB, NIDDK

COOPERATING UNITS *(if any)*

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Laboratory of Molecular Biology

SECTION

Section on Physical Chemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8	8	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have continued our studies of chromatin structure, in order to learn how DNA is packaged within eukaryotic nuclei, and in particular to learn how that structure is altered in the neighborhood of genes that are being expressed. We have studied the way in which chromatin containing expressed genes folds to form compact fibers, and we have shown that at high salt concentrations such chromatin is not capable of the full compaction that occurs in inactive chromatin. We have also continued our studies of the structure of chromatin in the neighborhood of the chicken adult β globin gene, isolated from the nuclei of erythrocytes in which the gene is expressed. We had earlier identified sites within the nuclease hypersensitive domain in the 5' flanking region of the active gene that are binding sites for DNA sequence-specific proteins which we had partially purified. We have now shown that there are at least 3 different proteins involved, each of which binds to a distinct portion of the 5' flanking region, and we have studied their appearance in red cells as a function of developmental stage. A similar analysis has been undertaken for one of the α globin genes. In order to determine the biological function of these and other globin DNA sequences, we have developed a method for transfecting DNA into primary chicken erythrocytes at various stages of development. The method makes use of controlled, specific red cell lysis to obtain high levels of expression of transfected DNA. The method has led to the detection of a new regulatory region with the properties of an enhancer in the 3' flanking region of the β globin gene. The region with enhancer activity is the site of another hypersensitive domain we had previously identified. We have identified specific protein factors that bind to the region, and we have used footprinting methods to determine the binding sites precisely.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 34002-22 LMB
Formerly
Z01 AM 34002-21 LMB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Enzyme Structure

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	David R. Davies, Chief, Section on Molecular Structure		LMB/NIDDK
Others:	T. Narayana Bhat	Visiting Associate	LMB/NIDDK
	Gerson H. Cohen	Research Chemist	LMB/NIDDK
	Craig Hyde	Staff Fellow	LMB/NIDDK
	Kaza Suguna	Visiting Fellow	LMB/NIDDK
	Eduardo Padlan	Expert	LMB/NIDDK

COOPERATING UNITS *(if any)*
Alexander Wlodawer, National Bureau of Standards, Gaithersburg, MD
Hugh Savage, National Bureau of Standards, Gaithersburg, MD
Warner E. Love, The Johns Hopkins University, Baltimore, MD

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	3.5	1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

1. High resolution X-ray diffraction data have been collected for an aspartyl protease from Rhizopus chinensis, a renin analog. Several renin inhibitors have been analyzed bound to this enzyme. The structure suggests a mechanism of action.

2. The X-ray diffraction analysis of tryptophan synthetase from Salmonella typhimurium has been continued. X-ray data to 2.8Å for the protein and four heavy atom derivatives have been measured and a preliminary analysis to determine the three-dimensional structure is underway.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 34003-18 LMB
Formerly
Z01 AM 34003-17 LMB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Three-Dimensional Structure of Proteins of the Immune System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	David R. Davies, Chief, Section on Molecular Structure		LMB/NIDDK
Others:	Gerson H. Cohen	Research Chemist	LMB/NIDDK
	Enid W. Silverton	Research Chemist	LMB/NIDDK
	Eduardo A. Padlan	Special Expert	LMB/NIDDK
	Steven Sheriff	Staff Fellow	LMB/NIDDK

COOPERATING UNITS *(if any)*
Sandra Smith-Gill, National Cancer Institute, NIH

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Laboratory of Molecular Biology

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Section on Molecular Structure

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	3.5	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

High resolution (2A) X-ray diffraction data have been measured for J539. This will greatly extend the structure analysis of this Galactan-building Fab.

High resolution X-ray data have been measured for the Fabs of two monoclonal antibodies to lysozyme complexed to lysozyme. A molecular replacement analysis of these structures is in progress.

Some structural factors relating to antibody assembly have been analyzed. In particular, the nature of the interaction between C_H1 and C_L has been studied. A proposed model for the structure of the IgE Fc has been constructed.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 35000-22 LMB
Formerly
~~Z01 AM 35000-21 LMB~~

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Chemical and Structural Investigations of Nucleic Acids and Related Molecules

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H. Todd Miles	Chief, Section on Organic Chemistry	LMB/NIDDK
	F. B. Howard	Research Chemist	LMB/NIDDK
	J. Frazier	Research Chemist	LMB/NIDDK
	Y. Hae Kim	Guest Worker	LMB/NIDDK
	C. Q. Chen	Visiting Fellow	LMB/NIDDK
	H. Miyashiro	Visiting Fellow	LMB/NIDDK

COOPERATING UNITS *(if any)*

Foreign: Girjesh Govil, Tata Inst. Fundamental Res., Bombay
Yong Hae Kim, Korea Advanced Inst. Sci. & Tech., Seoul
U.S.: J. Cohen, National Cancer Institute, NIH

LAB/BRANCH

Laboratory of Molecular Biology

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Section on Organic Chemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.5	4.5	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our investigations of the chemistry and structure of nucleic acids and related molecules have continued. Current studies and results include the following:

Poly 2-amino-8-methyldeoxyadenylic has been prepared and its physical properties studied. The opposing effects of the two substituents on heteroduplex formation are interpreted in terms of their influence on conformational equilibria of the polymer. Marked contrast of these effects in the ribo and deoxy series has been observed.

High salt concentrations convert poly ($2NH_2A$-dBrU) and poly ($d2NH_2A$-dIU) to a different conformation, reported in the literature to be a left-handed Z form. 2D NMR studies, in collaboration with J. S. Cohen and B. Borah of NCI, however, have clearly shown that the high salt conformation is A and not Z.

The above A $\rightleftarrows$ B conversion was found to be temperature dependent. The enthalpy of the process and ion release per nucleotide were determined.

Solid phase cyanoethylphosphoramidite methods were used to synthesize DNA oligomers containing restriction endonuclease recognition sites. These will be used for physical studies.

A 2D NMR study of GAATTCGAATTC containing the Eco RI site has been carried out with G. Govil (TIFR, Bombay). Conformational details of the sugar pucker, glycosidic angle, and secondary structure have been obtained.

Spectroscopic studies of the helix-forming nucleoside, isoguanosine, have led to proposal of a novel structure for the helix, at variance with those suggested in the literature.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 35050-15 LMB
Formerly
Z01 AM 35050-14 LMB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Replication, Recombination and Repair of Microbial DNA

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. Tomizawa	Chief, Section on Molecular Genetics	LMB/NIDDK
Others:	S. Dasgupta	Visiting Associate	LMB/NIDDK
	H. Masukata	Visiting Associate	LMB/NIDDK
	S. Nakasu	Visiting Fellow	LMB/NIDDK
	M. Brenner	Expert	LMB/NIDDK

COOPERATING UNITS *(if any)*

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INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	4.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies on DNA replication of plasmid ColE1 have been continued. Transcripts (RNA II) that start 555 nucleotides upstream of the replication origin by RNA polymerase form a hybrid with the template DNA. The hybridized transcript is cleaved by ribonuclease H at the origin and used as the primer for DNA synthesis by DNA polymerase I.

Primer formation is affected by point mutations. Each of them affect a specific stage of primer formation and inactivated primer. Studies on RNA II structure by a computer program under constraints which are based on biochemical and genetic data show that functional RNA II has a unique secondary structure that fold in a specific tertiary conformation.

Primer formation is regulated by a plasmid-specified small RNA (RNA I). RNA I binds to RNA II at the complementary region. This binding results in inhibition of formation of the secondary structure necessary for primer formation. Binding starts by reversible interaction between loops of folded structures. This interaction facilitates stable binding that initiates at the 5'-end of RNA I.

Primer formation is also regulated by a 63-amino acid protein (Rom) specified by the plasmid. The protein stimulates binding of RNA I to RNA II by affecting initial reversible interaction of these RNAs. Thus the protein enhances the inhibitory action of RNA I. Inhibition of primer formation by RNA I in the presence or absence of the protein determines the copy number of a plasmid in a cell and the incompatibility between related plasmids.

GPO 914-916

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 36002-12 LMB
Formerly
Z01 AM 36002-11 LMB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Novel Recombination Systems

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J. L. Rosner, Research Biologist LMB/NIDDK

Others: R. Khanna Visiting Fellow LMB/NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Section on Microbial Genetics

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use stenderd unreduced type. Do not exceed the space provided.)*

The location of the pgl (phosphogluconolactonase) gene of Escherichia coli on a 5.8 kb Kpn I fragment has been determined by insertional mutagenesis. The gene encodes a 42 KD protein, as observed in maxicell preparations. sapA, a gene involved in transpositional activation of the cryptic bgl operon, appears to be identical to pgl since mutations that inactivate one function inactivate the other and results in the loss of expression of the 42 KD protein.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 36003-02 LMB
Formerly
~~Z01 AM 36003-01 LMB~~

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Nonheritable Antibiotic Resistance

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J. L. Rosner, Research Biologist, LMB/NIDDK

Others: R. Hauptschein Student Volunteer LMB/NIDDK
D. Murray Biologist LMB/NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Section on Microbial Genetics

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.9	.6	.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our previous work showed that certain weak acids, especially aspirin and salicylate, induce nonheritable resistance to ampicillin, chloramphenicol, nalidixic acid and tetracycline in Escherichia coli. Similar results have now been obtained in various other bacteria including certain Bacillus, Klebsiella, Salmonella, Shigella and Vibrio, but not Streptococcus or Staphylococcus species. Furthermore, acetaminophen (Tylenol) has also been found to induce noninheritable drug resistance E. coli.

Several additional effects of salicylate on E. coli have been demonstrated. In the presence of salicylate, the bacteria require methionine or isoleucine and valine, appear to be Lac^-, and are hypersensitive to kanamycin. Mutants that do not show these traits are being isolated and characterized.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 36051-18 LMB

Formerly

Z01 AM 36051-17 LMB

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Origins of Mammalian DNA Replication in Normal and SV40 Transformed Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert G. Martin,	Chief, Section on Microbial Genetics	LMB/NIDDK
Others:	R. L. Lechner	Senior Staff Fellow	LMB/NIDDK
	B. S. Rao	Visiting Fellow	LMB/NIDDK
	E. Karawya	Visiting Associate	LMB/NIDDK
	S. S. Wang	Research Chemist	LMB/NIDDK
	H. Manor	Senior Research Scientist	LMB/NIDDK

COOPERATING UNITS *(if any)*

B-K. Tye, Cornell University, Ithaca, New York

Foreign: M. Zannis-Hadjopoulos, McGill Cancer Center, Montreal, Canada

G. Kaufmann, Tel Aviv University, Tel Aviv, Israel

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Laboratory of Molecular Biology

SECTION

Section on Microbial Genetics

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.5	5.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have isolated a number of cloned monkey DNA fragments, some of which are believed to contain origins of DNA replication. Two of the fragments contain a class of moderately reiterated highly dispersed sequences known as the "O-family". We have been attempting to isolate a sequence-specific binding protein to these fragments.

Another of the fragments contains the concensus sequence thought to be necessary for providing origin function for replication in yeast (the autonomously replicating or "ARS" sequence). The fragment has been cloned in yeast tester plasmids and does appear to have very weak ARS functions.

We have sequenced a large portion of monkey mitochondrial DNA and compared it with the human sequence.

We have prepared monoclonal antibody to DNA molecules containing cruciform structures. We have shown that the monoclonal antibody protects against the action of Endo VII but not single-stranded nucleases, suggesting that the antibody recognizes the base of the cruciform structure.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 36101-12 LMB
(formerly Z01 AM 36101-11 LMB)

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Energy Conversion in Biology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Terrell L. Hill, Chief, Section on Theoretical Molecular Biology, LMB, NIDDK

Others: Y. Chen, R.C., LMB/NIDDK
H.V. Westerhoff, V.A., LMB/NIDDK
F. Kamp, V.F., LMB/NIDDK
R.W. Hendler, Sect. Chief, LCB/NHLBI
P.B. Chock, Sect. Chief, LB/NHLBI
R.D. Astumian, S.F., LB/NHLBI
J.G. Koster, G.R., NICHD
E.D. Korn, R.B., LC/NHLBI
D. Pantaloni, V.S., LC/NHLBI
M.F. Carlier, V.S., LC/NHLBI

Cooperating Units
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F. Ferrer, Univ. Aut. Barcelona, Spain
T.Y. Tsong, Johns Hopkins Sch.Med., Baltimore
K. van Dam, Univ. Amsterdam, Netherlands
D.B Kell, Univ. Col. Wales, Great Britain
J.A. Berden, Univ. Amsterdam, Netherlands
R.L. van der Bend, Netherlands Cancer Institute, Amsterdam, Netherlands
O.H.J. Destree, Hubrecht Lab, Utrecht, Netherlands

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Theoretical Molecular Biology

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.6	3.6	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A larger number of different topics have been studied in the general field of free energy transduction. The most important areas in which progress has been made are the dynamics of actin and microtubule assembly and disassembly, the potential role of non-stationary electric fields in biological free-energy transduction, the theory of the control of microbial growth, the spatial profile of electric potentials across biological membranes, assay methods for channelled metabolism, and role of protons in chemiosmotic systems.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 36102-15 LMB

(formerly Z01 AM 36102-14 LMB)

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Statistical Thermodynamics of Protein and Polynucleotide Systems

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: T. L. Hill Chief, Section on Theroretical Molecular Biology, LMB, NIDDK

OTHERS: Y. Chen Research Chemist LMB, NIDDK

H. V. Westerhoff Visiting Scientist LMB, NIDDK

COOPERATING UNITS *(if any)*

A. Maxwell, University of Leicester, U.K.

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Theoretical Molecular Biology

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A statistical mechanical study has been made of a one-dimensional piggy-back binding model and its application to polymer-activated enzyme reactions. In particular, the ATP hydrolysis rates of DNA gyrase in the presence of long DNA molecules has been investigated.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 36104-05 LMB
Formerly
Z01 AM 36104-04 LMB

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Thermal Measurements of Biomolecular Systems

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P.D. Ross	Research Chemist	LMB, NIDDK
Others:	A.C. Steven	Visiting Scientist	LPB, NIDDK
	T. O'Leary	Medical Staff Fellow	CDB, DBB
	I. Levin	Chemist	LCP, NIDDK

COOPERATING UNITS *(if any)*

Andrew Shrake, CDB, DBB
Lindsay W. Black, University of Maryland Medical School, Baltimore, Maryland
Foreign: None

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Section on Physical Chemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Thermodynamic characterization using high sensitivity differential scanning calorimetry has been carried out in the following areas:

Phospholipid Membranes: The effects of isomeric unsaturated long-chain fatty alcohols upon the pretransition of phospholipid liposomes has been interpreted in terms of the effects of these perturbants upon head chain tilt and in terms of the depth of penetration of the alcohol acyl chains in either stabilizing or destabilizing the bilayer.

Proteins: The complex, i.e. either asymmetric or polymodal, thermograms obtained in differential scanning calorimetry under conditions of subsaturating ligand concentrations has been accounted for by a model based upon the Law of Mass Action and LeChatelier's principle.

Macromolecular Assembly Processes: The complex thermal denaturation profile of the bacteriophage T4 head particle in the small unexpanded form has been identified. The increase in thermal stability arising in the transformation to the expanded lattice form producing large particles has been described.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 36105-04 LMB
Formerly
Z01 AM 36105-03 LMB

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Influences of Macromolecular Crowding on Biochemical Systems

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: S.B. Zimmerman Research Chemist LMB, NIDDK

Others: B. Harrison Research Chemist LMB, NIDDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Section on Physical Chemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

T4 polynucleotide kinase rapidly loses activity during its reaction on duplex DNA termini. Addition of high concentrations of nonspecific polymers reverses or prevents this inactivation. In contrast, additions of related materials of lower molecular weight are relatively ineffective in stabilizing the kinase. Such a pattern suggests that the stabilizing effects of polymers on kinase activity are due to macromolecular crowding. An effect of crowding on the known tendency of the kinase to undergo oligomerization reactions is consistent with our observations. This effect of polymers on the kinase can be exploited to greatly increase the amount of reaction obtainable on 5'-hydroxyl groups of duplex DNA substrates located at recessed or blunt ends or at "nicks" which are otherwise difficult to effectively phosphorylate or dephosphorylate.

Macromolecular crowding was found to cause no striking change in the processivity of the T4 DNA ligase under several reaction conditions.

Interactions with the Patent Office in connection with an application on behalf of the Government have been successfully concluded and we await the issuance of a patent based on our previous studies of polymer-stimulated ligation of DNA.

ANNUAL REPORT OF THE METABOLIC DISEASES BRANCH
National Institute of Diabetes and
Digestive and Kidney Diseases

The general goals of the Branch are to investigate the mechanism of action of hormones controlling ion transport and mineral metabolism and to investigate the immunological and pathological factors mediating kidney disorders. The branch currently includes sections of Molecular Pathophysiology (Dr. Spiegel), Mineral Metabolism (Dr. Marx), Endocrine Regulation (Dr. Aurbach) and Kidney Disease (Dr. Balow). Integration of these sections is related to common interests in the pathophysiology of metabolic disorders which interface with the kidney. Systems under study include renal and skeletal tissue, transgenic mice, isolated cells (kidney and parathyroid) in culture, hormone receptors (β adrenergic, parathyroid hormone, and 1,25 dihydroxy vitamin D), parathyroid cell growth factors, regulatory proteins of the adenylate cyclase complex, and T cell and B cell function in disorders of immunoregulation.

Analysis of Hormone Receptor

Interactions of catecholamines with adrenergic receptors and activation of adenylate cyclase are under study with the plasma membranes of several cell systems. Specific receptors have now been identified on turkey erythrocytes, parathyroid cells, pineal cells, rat, guinea pig and monkey lung membrane preparations, rat osteosarcoma cells and rat liver membranes. Control of receptor accumulation in isolated cell culture systems in vitro are being studied with a view toward gaining knowledge about the molecular biology of receptors and how they are linked to intracellular response systems.

Receptors for Parathyroid Hormone

Studies in collaboration with Dr. T. Murray (St. Michael's Hospital, Toronto) have led to development of radiolabeled intact bovine parathyroid hormone as a ligand. The radiolabeled agonist binds to receptors in canine renal plasma membranes or in cultured osteosarcoma cells with kinetic properties distinct from those of radiolabeled synthetic amino terminal bioactive fragments.

The radioiodinated parathyroid hormone binds to specific receptors on cultured rat osteosarcoma cells and interaction with these receptors correlates well with stimulation of cyclic AMP production in this cell system.]

Guanine Nucleotide Binding Proteins (G-proteins)

A family of G-proteins acts to transduce signals across cell membranes. Identified G-proteins include: a) G_s and G_i, the stimulatory and inhibitory G-proteins, respectively, associated with adenylate cyclase; b) transducin (TD) -the G-protein of rod photoreceptor outer segment membranes; c) G_o - a G-protein of unknown function, discovered in, and abundant in brain. Recent evidence suggests that G-proteins are also involved in coupling receptors to other effector systems including ion channels, and phosphatidylinositol breakdown.

We have: a) purified G-proteins for functional reconstitution studies; b) produced polyclonal antisera vs. specific G-protein subunits for immunochemical studies; c) cloned cDNA for G-protein alpha subunits for determination of primary structure and other molecular biologic studies; a brief summary of the major findings in each area follows:

A. Reconstitution studies - Receptor - G-protein interactions show relative, but not absolute specificity. For the β-adrenergic receptor, the order of preference in coupling is $G_s > G_i >> TD$; for rhodopsin, the order is $TD = G_i = G_o >> G_s$; for the α-2-adrenergic receptor, $G_i = G_o > TD >> G_s$.

Studies with G-proteins and the catalytic unit of adenylate cyclase show that the β/gamma-S subunit rather than the G_i-α subunit is the major inhibitor, and that inhibition involves stabilization of the non-dissociated form of G_s. Subtle differences in inhibitory efficiency between TD-β/gamma and brain G-β/gamma may reflect the differences in the gamma subunits.

B. Immunochemical studies - Specific antibodies vs. TD-α, G_o-α, G_i-α, and the common G-β subunit have been used in immunoblots and immunocytochemistry. Among the results obtained: 1) TD-α immunoreactivity is detected in rods but not cones; we speculate that the latter may have a unique form of transducin. 2) The quantity of G_i and G_o undergoes dramatic changes during differentiation of 3T3-L1 fibroblasts to adipocytes. 3) Human neutrophils and rat CL6 glioma cells contain high concentrations of a pertussis toxin substrate. Immunoblot assays for the known pertussis toxin substrates G_i, G_o, and TD, suggest that a novel G-protein is present in these cells.

C. Molecular biologic studies - Multiple cDNAs for G-α subunits have been cloned from a human brain library. Most are related to G_s-α but several code for G_i-α. Complete DNA sequencing of several clones has been completed. The results show that the human DNA sequence is 95% homologous to the bovine sequence. Conservation is not limited to the coding region, but is just as high in the 3' untranslated region. The latter may reflect an important regulatory function. Multiple forms of G_s-α cDNA occur. The pattern of variation is consistent with multiple mRNA's resulting from alternative splicing of the $G_s\alpha$ gene. S1 nuclease protection experiments provide evidence for the existence of multiple G_s-α mRNA species. [Drs. Spiegel, Carter, Goldsmith].

Pseudohypoparathyrodism (PHP)

Subjects with PHP and the phenotypic features of Albright's hereditary osteodystrophy (AHO) generally show resistance to multiple hormones. Previous studies identified the molecular defect in such subjects (PHP Ia) as a

deficiency in the activity of the stimulatory guanine nucleotide binding protein (G_s) associated with adenylate cyclase. Using cloned cDNA probes for the alpha (guanine nucleotide binding) subunit of G_s, we now find reduction in steady-state mRNA levels in Northern blots of total fibroblast RNA from subjects with PHP Ia compared with normal subjects. These studies show that reduced synthesis of G_s-alpha is the likely basis for deficient G_s activity in PHP Ia. Studies in progress, including cloning of genomic DNA for G_s-alpha and analysis of Southern blots from subjects with PHP Ia, should elucidate the molecular basis for this inherited form of hormone resistance. [Drs. Spiegel and Carter, NIDDK].

Primary Hyperparathyroidism and Familial Hypercalcemia

Clinical studies are continuing on primary hyperparathyroidism and its familial variants. Detailed family screening and case findings has produced approximately 65 kindreds for analysis. These studies have allowed segregation of the commonest familial variants into two apparently distinct disease syndromes - familial multiple endocrine neoplasia type 1 (FMEN I) and familial hypocalciuric hypercalcemia (FHH). FHH was distinguished from FMEN I by 1) virtually a 100% penetrance for hypercalcemia before age 20, 2) milder clinical manifestations - low incidence of recurrent nephrolithiasis or recurrent peptic ulceration, 3) no hypercalciuria, 4) normal basal concentrations of gastrin, and 5) poor response to subtotal parathyroidectomy. Distinction between the two syndromes, both inherited as autosomal dominant traits, is important because in FHH the clinical course is generally milder and subtotal parathyroidectomy is less likely to be beneficial. FHH accounts for approximately 10% of all unsuccessful parathyroidectomies in hypercalcemia. In FHH the ionized and ultrafiltrable calcium concentration in serum are elevated in proportion to the increase in total calcium. In these patients the filtrable load of calcium is high in association with a marked decrease in renal calcium clearance. Even when these patients become surgically hypoparathyroid, the low renal clearance of calcium is strikingly persistent during calcium infusion. The concentration of parathyroid hormone in plasma is lower in patients with FHH than in typical primary hyperparathyroid patients with similar degrees of hypercalcemia whether assessed by PTH radioimmunoassay or by renal clearance of cAMP or phosphate. The parathyroid glands show hyperplasia in most cases. In several kindreds one or more members have exhibited life-threatening primary hyperparathyroidism in the neonatal period. This may result sometimes from a double dose of the FHH gene. Dispersed parathyroid cells from one severely affected neonate showed a striking decrease in sensitivity of PTH secretion to extracellular calcium [Drs. Marx, Spiegel, Fitzpatrick, Bliziotes, and Aurbach].

Familial multiple endocrine neoplasia type 1 (FMEN1) is an autosomal dominant disorder characterized by hyperfunction of parathyroids, pancreatic islets, and anterior pituitary. Affected organs show features suggestive of increased proliferation. Virtually all subjects expressing the gene show primary hyperparathyrodism. Primary hyperparathyroidism is usually first recognizable between ages 20-40, and it shows a high recurrence rate after subtotal parathyroidectomy (approximately 50% after 10 years). We have evaluated multiple indices for use in screening in a very large kindred. We tested 221 members and newly identified 16 as carriers. Albumin-adjusted

calcium and PTH were most useful; gastrin and prolactin analyses were not useful for screening but showed promise in followup of known carriers. With cultured bovine parathyroid cells, we found abnormally high mitogenic activity in plasma from 23 of 27 subjects with FMEN1. Well-characterized growth factors or known parathyroid secretagogues showed far less parathyroid mitogenic activity than these FMEN1 plasmas. The mitogenic factors(s) appear to be a protein of 50,000 mw. We have begun purifying this factor for further characterization. [Drs. Brandi, Fitzpatrick, Aurbach, Goldsmith, Spiegel, Bliziotes, Nanes, Marx).

Studies on noninvasive and invasive modes of localizing parathyroid tumors continue. Parathyroid adenoma localization has been evaluated using the new non-invasive magnetic resonance imaging technique (Drs. Aurbach, Marx, Spiegel, Bliziotes, Nanes, Fitzpatrick, NIDDK; Dr. Doppman, diagnostic Radiology). Initial results were disappointing but the accession of a specialized neck collar has led to better resolution in the paratracheal and mediastinal areas. Patients are currently under evaluation with this new technique as it compares to thallium-technetium scans, computed tomography, and ultrasound. A high degree of success has been obtained in localizing tumors through vascular catheterization procedures. Parathyroid arteriography developed and performed by Dr. John Doppman afforded, in approximately 45% of cases tested, the identification of abnormal masses of tissue subsequently identified as parathyroid. In the most difficult cases, localization of parathyroid tissue can be aided by identifying high concentrations of parathyroid hormone by radioimmunoassay in veins draining the lesion. Fine needle aspiration is another new method that can obviate other invasive localization procedures. We have aspirated with guidance by computerized tomography or ultrasound approximately 20 such lesions that were subsequently confirmed surgically as parathyroid. RIA of the aspirates showed high concentrations of PTH in all but one. Eight mediastinal adenomas have been treated nonsurgically by percutaneous injection via catheter of occlusive agents into the arterial blood supply with 7 complete and one partial remissions. [Drs. Aurbach, Marx, Spiegel, Fitzpatrick, Bliziotes, Nanes, NIDDK: Dr. Norton, NCI, Drs. Doppman and others, Diagnostic Radiology, CC].

A new series of radioligands for parathyroid hormone radioimmunoassay has been developed, using synthetic sequences and analogs from the midregion of human PTH. These reagents have led to development of sensitive and practical radioimmunoassays. Using these assays and gel filtration of plasmas, we have demonstrated that certain hyperfunctioning parathyroid glands release a previously unrecognized large PTH fragment containing the midregion but deficient in carboxyterminal immunoreactivity. [Dr. Marx, Mr. Sharp].

Rapid determination of intraoperative UcAMP excretion (using the Gammaflo machine for 7 min cAMP radioimmunoasay) has proven to be a valuable tool in guiding surgery for primary hyperparathyroidism, particularly in patients with multigland disease. Persistent elevation of UcAMP requires continued search for abnormal tissue even after 1 or more abnormal glands have been removed. A rapid (mean 1.5 hours) drop in UcAMP to the normal range obviates the need for continued exploration even in cases where histologic confirmation of parathyroidectomy is lacking. Spurts in UcAMP above baseline may provide a clue to the location of abnormal parathyroid tissue. [Drs. Spiegel, Marx, Fitzpatrick, Bliziotes, Nanes, and Aurbach, NIDDK: Dr. Norton, NCI Surgery].

Determination of urinary cAMP excretion postoperatively in patients undergoing neck exploration for primary hyperparathyroidism is a useful method for assessing postoperative parathyroid function. UcAMP excretion declines postoperatively in all patients in whom hypercalcemia is corrected but not in those with persistent hypercalcemia. In patients becoming severely hypocalcemic (and requiring vitamin D therapy) postoperatively, UcAMP measurement enables one to distinguish patients with decreased parathyroid reserve as the cause for hypocalcemia (low UcAMP excretion) from patients with healing osteitis fibrosa ("hungry bones" with secondary hyperparathyroidism) as the basis for hypocalcemia. UcAMP in the latter group is often elevated but can be suppressed if serum calcium is normalized. Elevated UcAMP excretion postoperatively in the face of hypocalcemia enables one to predict that vitamin D therapy will be required temporarily (if at all) and precludes the need for parathyroid autografts. [Drs. Spiegel, Marx, Fitzpatrick, Bliziotes, and Aurbach, NIDDK].

Postoperative patients with surgically corrected hyperparathyroidism are being actively evaluated in a five year follow up study (Dr. Udelsman, Norton NCI, Drs. Marx, Fitzpatrick NIDDK). These patients are being studied for sequalae such as hypoparathyroidism, recurrent hyperparathyroidism, and complications such as vocal cord paralysis.

Secretion of Parathyroid Hormone

Studies have continued with dispersed bovine and human parathyroid cells. PTH secretion from parathyroid glands in vivo and cells in vitro is controlled by intracellular calcium and cyclic AMP. Control by calcium is altered in certain pathologic states (glandular adenomas, carcinomas and perhaps hyperplasia). Agents that alter cellular cAMP change PTH secretion in the same direction. Calcium decreases cellular cAMP, but most of its effect to inhibit secretion is independent of changes in cellular cAMP.

Intracellular calcium measurements using Quin 2 fluorescence indicate that $PGF_{2\alpha}$ causes a decrease in intracellular calcium. This is blocked by pertussis toxin treatment of parathyroid cells. [Drs. Aurbach, Fitzpatrick, Brandi].

Calcium inhibition of parathyroid hormone secretion was evaluated utilizing pertussis toxin as a probe. Pertussis toxin catalyzes ADP-ribosylation and inactivation of the inhibitory guanine nucleotide regulatory protein, N_i. Studies in dispersed bovine parathyroid cells indicates that calcium inhibition of parathyroid hormone secretion is mediated via N_i. Further studies with calcium channel agents show that calcium channels are involed in regulation of PTH secretion. Two enantiomers, (+)202-791 and (-) 202-791, were supplied by Sandoz, Ltd, Basle. The former is a calcium channel agonist and the latter, a calcium channel antagonist. The agonist (opens calcium channels, facilitating Ca entry) inhibits secretion. The antagonist stimulates secretion. Studies with pertussis toxin indicate that calcium channel regulation of secretion is linked through a guanine nucleotide regulatory protein [Drs. Fitzpatrick, Brandi, and Aurbach].

The inhibitory effects of α-adrenergic agonists and $PGF_{2\alpha}$ were studied extensively in bovine parathyroid cells. Pertussis toxin releases the inhibition of these agents on PTH secretion and cAMP accumulation indicating that their actions are mediated via N_i. Studies in abnormal human tissue have also reflected different mechanisms that will be explored further.

A bovine parathyroid cell culture line has been established to study growth of cells and secretion therefrom. Cell culture characteristics differ from the in vitro models previously used. This system is proving to be a valuable in vitro model to study factors that stimulate or inhibit growth. Use of this cell system has facilitated identification of a parathyroid cell growth factor circulating in familial multiple endocrine neoplasia type I. In autoimmune hypoparathyroidism, an IGM has been found that causes complement-dependent cytotoxicity in parathyroid cells. [Drs. Aurbach, Brandi, Fitzpatrick, Sakaguchi, Marx].

Vitamin d Resistance and Related Disorders

The role of $1,25(OH)_2D3$, the most potent natural metabolite of vitamin D, has been assessed in hypocalcemic states. This very rapidly acting drug has simplified the management of hypocalcemia following parathyroidectomy: during this time skeletal remineralization imposes large but rapidly diminishing requirements for calcium.

We have evaluated patients with extreme resistance to $1,25(OH)_2D$. This can be a transient state as following parathyroidectomy or a permanent state as in familial cases. We have evaluated 20 patients with familial resistance to $1,25(OH)_2D$. Most patients have hypocalcemic rickets, usually with associated total alopecia. Mineral homeostasis is usually improved by treatments that sustain $1,25(OH)_2D$ levels at 10-100 times normal.

Specific intracellular defects have been evaluated using cultured skin fibroblasts from these patients. With skin fibroblasts cultured from normals, a typical $1,25(OH)_2D$-receptor can be identified by binding in soluble extracts, by nuclear uptake of hormone with intact cells, or by elution of occupied receptor from DNA-cellulose. Fibroblasts from patients with familial resistance to $1,25(OH)_2D$ have shown a spectrum of defects including nonfunctional receptors, diminished numbers of receptors, and apparently normal receptors. Among cases with normal hormone binding sites on the receptors some show receptors with deficient binding to nucleus while others show normal binding to nucleus but abnormal interaction with nonspecific DNA (as DNA-cellulose). In one patient, osteoblast-like cells from bone biopsy exhibited a defect analogous to that in skin fibroblasts of the same patient. Even when receptors have unmeasurable hormone-binding activity, the receptor protein has been present in normal amounts according to immunoassay suggesting point mutations in the hormone-binding region. Cellular action of $1,25(OH)_2D3$ can be analyzed by measuring its induction of the 25(OH)D 24-hydroxylase enzyme system. Cultured skin fibroblasts from all patients with hereditary resistance to $1,25(OH)_2D$ exhibit defects in this induction. [Drs. Marx, Bliziotes, Barsony, Brandi, Nanes, Liberman, MDB, NIDDK; Dr. Eil, BNMC; Drs. Pike and Haussler, U. Arizona].

New world primates show resistance to many steroid hormones, including $1,25(OH)_2D$. EB virus transformed B lymphocytes from a new world primate showed receptors with lower affinity and capacity for $1,25(OH)_2D3$ then in similar cells from old world primates (human or macaque) [Drs. Marx, Bliziotes, Liberman, NIH].

KIDNEY DISEASES

Studies of the immunopathogenesis and the treatment of lupus nephritis and other forms of glomerulonephritis are the focus of the Kidney Disease Section. Ongoing collaboration with the Arthritis and Rheumatism Branch, NIAMS, is an important element in the conduct of several of the clinical studies. Therapeutic studies from our group have provided the first compelling evidence the cytotoxic drug therapy is superior to conventional corticosteroid therapy in preventing end-stage renal failure in lupus nephritis.

I. Lupus Nephritis (J. Balow)

A. Immunopathogenesis. Murine models are being utilized to investigate the different forms and components of lupus nephritis. The immunologic characteristics of the immune complex deposits and the lymphoid cell infiltration are being dissected by immunohistologic and electron microscopic techniques. Differences among the strains promise to enhance our understanding of the diverse manifestations and response to treatment of human lupus nephritis (Austin, Balow).

B. Immunoregulatory Studies. A multiplicity of T and B lymphocyte abnormalities have been found in patients with SLE. Heightened and poorly regulated B cell activity is characteristic of SLE. Defective T suppressor cell activity was found to be present in some but not all cases of active SLE. Moreover, T cytotoxic cell and natural killer cell activities are deficient and could permit the emergence of abnormal and unregulated autoantibody producing cells. An alternative immunoregulatory defect leading to excessive B cell activity has been noted in certain lupus mouse strains, namely, T helper cell hyperactivity. Our group has found increased numbers of circulating T cells bearing activation markers and proto-oncogene expression which function to increase immunoglobulin secretion by autologous B cells. It will be important to delineate whether different mechanisms underlie the heightened production of antibodies by B cells in different patients with lupus nephritis (Tsokos, Inghirami, Smith, Balow).

C. Therapeutic Studies. Current protocols are designed to increase and refine the therapeutic index of different immunosuppressive drugs for lupus nephritis. Studies to date have shown that cytotoxic drugs are superior to conventional prednisone therapy and that intermittent high-dose therapy maintains efficacy while reducing toxicity. Patients with severe lupus nephritis are being intensively treated with pulse methylprednisolone or pulse cyclophosphamide to compare these two types of drugs and also to assess whether intensity or duration of cyclophosphamide is more important in stabilizing the renal disease. Laboratory studies of lymphoid cell modulation by the different drug regimens are ongoing in order to improve monitoring, drug administration and efficacy (Balow, Austin, Webb and members of ARB, NIAMS).

II. Glomerulonephritis (J. Balow)

A. Nephritic Factors. Patients with membranoproliferative glomerulonephritis and lupus nephritis develop autoantibodies to complement converting enzymes which cause abnormal consumption of complement components. These nephritic factors may participate in the pathogenesis of the renal diseases, but studies of their exact role has been hindered by lack of substantial quantities of pure preparations. Epstein-Barr virus transformed and sustained B cell lines which actively produce nephritic factors have been produced. One line from a patients with membranoproliferative glomerulonephritis secretes an IgG antibody which binds and stabilizes the alternate pathway C3 convertase enzyme. Another from a patient with lupus, binds the classical pathway C3 convertase. Nephritic factors with these functional activities correspond to known abnormalities of complement activation through the different pathways in these diseases. Studies of the binding sites, turnover and modulation of these autoantibodies are continuing (Tsokos, Hiramatsu, Balow).

B. Complement in Immune Regulation. Abnormal levels of complement components and deposition in sites of immunological reactions are characteristic of several forms of glomerulonephritis. The interactions of complement components and activation products with receptors on lymphoid cells are being studied to gain new insights into their potential role in lupus nephritis, membranoproliferative glomerulonephritis and other renal disorders. The precise role of complement receptors on B cell may be particularly relevant to the appearance of autoantibodies associated with these diseases. Studies are underway to determine the mechanism of the modulation of B cell responses through interaction of the complement receptor with natural complement ligands, Epstein-Barr virus and monoclonal antibodies (Tsokos, Thyphronitis, Balow).

C. Crescentic Glomerulonephritis. Rapidly progressive glomerulonephritis with severe crescent formation with two different immunologic basis are being studied. Goodpasture's disease is caused by autoantibodies to glomerular basement membrane. Crescentic glomerulonephritis without significant antigen-antibody deposits is of unknown pathogenesis but may be caused by cell mediated immune injury. Studies of abnormalities of systemic immune responses and characterization of local immune cell phenotypes within the renal lesions are being investigated. Therapeutic studies of these severe glomerular diseases include trials of immunosuppression alone or combined with plasmapheresis in Goodpasture's disease and a comparison of intensive pulse methylprednisolone versus cyclophosphamide in patients with idiopathic crescentic glomerulonephritis (Balow, Austin, Webb).

Studies of the mechanisms of end-stage glomerular disease

This laboratory has been interested in the cellular mechanisms leading to the development of glomerular scarring. The hypothesis is that resident glomerular cells play a key role in glomerular sclerosis which is favored by an increase in transcapillary pressure. This is being studied

using both in vivo and in vitro approaches. The work focuses on cell-cell interaction and their various responses to growth factors in health and disease.

III. Glomerulosclerosis (G. Striker)

A. Transgenic mice. We have identified several lines of mice transgenic for the early region of simian virus 40 that develop glomerular abnormalities which resemble those seen in human focal glomerulosclerosis and are proteinuric. We are looking in vivo by autoradiography for anomalies in glomerular cell behavior, i.e. proliferation, which would be expected to occur in a T antigen expressing cell. We have isolated several lines of glomerular endothelial, mesangial and epithelial cells from these mice. and have also cloned mesangial and epithelial cells from their normal littermates. Preliminary data indicate that proliferation is an early event in the development of glomerulosclerosis and are currently evaluating the growth factor(s) production in vitro (specifically P.D.G.F.) (MacKay, Striker, Striker).

B. Transfection of human glomerular cells. In order to study interaction between glomerular cells in vitro, we are developing lines of human glomerular cells. Primary outgrowth of human glomerular cells have been infected using a recombinant adeno-virus-SV40. We have obtained cells which are transfected as shown by positivity for T antigen. Epithelial and mesangial cells have been passaged multiple times. Establishment of stable human cell lines will allow study of glomerular functions in health and disease (Lange, Striker, Elliot, Striker, Bernstein).

C. Biology of insulin receptors in glomerular cells. We are currently studying the binding of insulin on glomeruli and glomerular cells from normal mice and humans. When these data are established, we will study the nature of the receptor and its possible modulation in mice who develop diabetes (N.O.D.) and glomerulosclerosis (transgenic mice). Mesangial cells from normal and transgenic mice express binding for radiolabelled insulin. These studies have just begun (Conti, Striker, MacKay, Striker, Lesniak).

IV. Studies of the Regulation of Glomerular Pressure (K. Bernstein)

It has been suggested that elevated glomerular pressure leads to glomerulosclerosis. The regulation of angiotensin converting enzyme (ACE) production plays a central role in maintaining normal glomerular pressure. This production is elevated in diabetes. This project is designed to isolate the gene encoding the enzyme to further study its regulation and expression, using cultured glomerular endothelial cells as a model. RNA has been isolated from mouse kidneys by polysome precipitation. Active ACE was obtained from mouse kidneys and lungs. Using sepharose, we have bound various pharmacological inhibitors of ACE, and will use these columns to isolate the RNA coding for ACE from the polysomes (Bernstein).

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 43002-21 MD (formerly ~~Z01 AM 43002-20 MD~~)

PERIOD COVERED: October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 cherecters or less. Title must fit on one line between the borders.)*

Structure, Secretion and Mechanism of Action of Parathyroid Hormone

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principel Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	G.D. Aurbach, M.D.	Chief, MDB, NIDDK
OTHERS:	L.A. Fitzpatrick, M.D.	Medical Staff Fellow, MDB,NIDDK
	M.L. Brandi, M.D.	Visiting Fellow MDB,NIDDK
	K. Kakaguchi, M.D.	Visiting Fellow MDB,NIDDK

COOPERATING UNITS *(if any)*

Endocrine Unit, Massachusetts General Hospital
Gastroenterology Research Lab. - University of Western Ontario

LAB/BRANCH

Metabolic Diseases Branch

SECTION

Endocrine Regulation Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	2.25	1.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

There is still much to be learned about the nature and secretory control of circulating parathyroid hormone in disease. It is the purpose of this project to study the secretion, function, and mechanism of action of parathyroid hormone, its relationship to human disease, and to develop clinically useful tests for circulating parathyroid hormone. From these studies it is expected that one can understand the pathophysiology of certain metabolic diseases of bone and endocrine disturbances. The entire structures of bovine, porcine, rat and human parathyroid hormone have been determined. Synthetic polypeptides representing bovine rat and human parathyroid hormone have been synthesized. These molecules show all the biological properties of the native hormonal polypeptides. Highly sensitive radioimmunoassays for the hormone have been developed and are being modified further for improved clinical diagnostic parameters. Studies show that the mechanism of action of the hormone is mediated through direct hormonal activation of adenylate cyclase in bone and kidney. Isolated parathyroid cells and culture systems have been develped that allow studies on secretory control of parathyroid hormone, and provide test systems to elucidate the pathophysiology of certain hypoparathyroid and hyperparathyroid states.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43003-21 MD
(formerly
~~Z01 AM 43003-20 MD~~)

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies on the Mode of Action of Thyrocalcitonin

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

G.D. Aurbach, M.D. Chief, MDB, NIDDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.25	1	.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose is to study the interaction of calcitonin with its specific receptor target organs. The current investigations should provide further insight into the structure-function relationship in calcitonin. Calcitonin is a small polypeptide hormone and therefore lends itself well to studies using synthetic peptide fragments. The system is also useful for characterizing hormone receptors in kidney, bone and other tissues. Studies are in progress to characterize further the interaction of calcitonin with tissue receptors. It will also be of interest to solubilize the receptors and characterize them chemically.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43004-21 MD
(formerly Z01 AM 43004-20 M

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies on pseudohypoparathyroidism and related disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Allen M. Spiegel, M.D.	Chief, Molec. Pathophys. Sec.	MDB, NIDDK
Others:	Anthony Carter, Ph.D.	Senior Staff Fellow	MDB, NIDDK
	Regina Collins, Ph.D.	Research Geneticist	MDB, NIDDK
	Charlotte Bardin	Biological Lab Tech.	MDB, NIDDK

COOPERATING UNITS *(if any)* C. Silve, Hospital Necker, Paris France
Arnold Moses, Upstate Medical Center, Syracuse, New York
Neil Breslau, Dept. of Medicine, Southwestern Medical School, Dallas, TX
R. Downs, Medical College of Virginia, Richmond

LAB/BRANCH
Metabolic Diseases Branch

SECTION
Molecular Pathophysiology Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.50	1.50	

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In 1942 Albright and his associates described the features of a new clinical syndrome "pseudohypoparathyroidism" (PHP). Patients with this disorder differ from those with idiopathic hypoparathyroidism: they show characteristic constitutional features (Albright's hereditary osteodystrophy - AHO) and do not respond to exogenous parathyroid hormone (PTH). Subsequent to the original report, patients lacking the typical somatic features of AHO but resistant to endogenous and administered PTH have been described. In PHP, UcAMP (urinary cyclic AMP) does not increase normally in response to PTH administration. This indicated that there is a defective hormone receptor-adenylate cyclase complex in this disorder. We have now shown that many patients with PHP+AHO (PHP Ia) show an approximately 50% reduction in activity of G_s (the stimulatory guanine nucleotide binding protein associated with adenylate cyclase) in membranes from multiple tissues. G_s deficiency presumably accounts for resistance to multiple hormones in such patients. Patients with PHP without AHO show normal G_s activity (PHP Ib) and resistance only to PTH, and preliminary studies suggest a PTH receptor defect in such patients. Rare patients with PHP and AHO and multiple hormone resistance show normal G_s activity.

Using cloned human cDNA probes for the alpha subunit of G_s, we now find that steady state mRNA levels from fibroblasts of subjects with PHP Ia are reduced by approximately 50% compared with normals. Genomic blotting and other molecular biologic approaches are being used to define the genetic abnormality responsible for G_s deficiency in PHP Ia.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43005-21 MD
(formerly
Z01 AM 43005-20 MD)

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Guanine nucleotide binding proteins as receptor-effector couplers

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Allen M. Spiegel, M.D.	Chief, Molec. Pathophys. Sec.	MDB, NIDDK
OTHERS:	Anthony Carter, Ph.D.	Senior Staff Fellow	MDB, NIDDK
	Paul Goldsmith, PH.D.	Research Biologist	MDB, NIDDK
	Terrye Zaremba, Ph.D.	Senior Staff Fellow	MDB, NIDDK
	Charles Woodard	Biochemistry Lab Tech	MDB, NIDDK
	Cyrena Simons	Chemist	MDB, NIDDK
	Ruth Vinitsky	Microbiologist	MDB, NIDDK

COOPERATING UNITS *(if any)*
P. Bray, M. Nirenberg, (NHLBI); G. Milligan, W. Klee (NIMH); J. Falloon, J. Gallin (NIAID); R. Lefkowitz, M. Caron, R. Cerione, (Duke University, N.C.); Y. Zick, R. Sagi-Eisenberg, (Weizmann Institute, Israel)

LAB/BRANCH
Metabolic Diseases Branch

SECTION
Molecular Pathophysiology Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	4.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A family of guanine nucleotide binding proteins, G-proteins, function as receptor effector couplers. Two distinct G-proteins, G_s and G_i couple stimulatory and inhibitory hormone receptors respectively to adenylate cyclase. A homologous retinal protein, transducin, couples a light receptor, rhodopsin, to cGMP phosphodiesterase. G_o is a G-protein of unknown function recently discovered in brain. We have purified G-proteins, raised antibodies to them, and used these to study the structure, function, and distribution of specific G-proteins. We have also used recombinant DNA methods to isolate cDNA clones for G-protein subunits, to determine their DNA sequence and primary structure, and to study mRNA synthesis and structure.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK-43006-11 MD

(formerly Z01 AM 43006-10 M

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Study of Hyperparathyroidism: Etiology, diagnosis and treatment

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	G.D. Aurbach, M.D.	Chief, MDB, NIDDK
OTHERS:	S.J. Marx, M.D.	Chief, Min. Met. Sec. MDB, NIDDK
	A.S. Spiegel, M.D.	Chief, Molec. Pathophys. Sec, MDB, NIDDK
	L.A. Fitzpatrick, M.D.	MDB, NIDDK
	M.M. Bliziotes, M.D.	MDB, NIDDK
	M.S. Nanes, M.D., Ph.D.	MDB, NIDDK
	L. Weinstein, M.D.	MDB, NIDDK

COOPERATING UNITS *(if any)*

Radiology Department, CC; Surgery Branch, NCI; Digestive Diseases Branch, NIDDK

LAB/BRANCH

Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda,. MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.75	2.50	2.25

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The project goal is the evaluation and treatment of hyperparathyroidism. Patients with persistent or recurrent hyperparathyroidism are referred for evaluation and treatment. Hereditary hyperparathyroidism in particular is under investigation in the hopes of delineating hereditary molecular abnormalities in glandular regulation, as exemplified in the multiple endocrine neoplasia syndromes. Evaluation ranges from epidemiologic studies of families to in-house studies of patients and to in vitro analyses of excised tissue. Techniques currently being employed and improved include radioimmunoassay of parathyroid hormone, ultrasonography, radiothallium scanning, magnetic resonance imaging, CAT scanning, selective arteriograpy and selective venous sampling for parathyroid hormone, parathyroid gland cryopreservation and autotransplantation, and transcatheter parathyroid gland infarction.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43007-06 MD
(formerly
Z01 AM 43007-05 MD)

PERIOD COVERED

October 1, 1985 to January 1, 1986 - Terminated

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Study of humoral hypercalcemia of malignancy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Allen M. Spiegel, M.D.	Chief, Molecular Pathophysiology Sec., MDB, NIDDK
Arthur Santora, M.D.	Guest Worker, MDB, NIDDK
Caroline Silve, M.D.	Guest Worker, MDB, NIDDK
Jeff Norton, M.D.	Surgery Branch, NCI
Andrew Saxe, M.D.	Surgery Branch, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Metabolic Diseases Branch

SECTION

Molecular Pathophysiology Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.5	.5	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Hypercalcemia is a common and serious complication of cancer. In some patients, hypercalcemia occurs without direct tumor invasion of bone, presumably via a humoral mechanism. The identity of humoral factor(s) is not known, although PTH or prostaglandins may be the cause in some cases. The project goal is to identify factors responsible for humoral hypercalcemia of malignancy. This involves study of patients with this syndrome as well as an animal model for the syndrome, a Leydig cell tumor of Fischer rats.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 43008-05 MD (formerly Z01 AM 43008-04 MD)

PERIOD COVERED: October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Vitamin D Resistance and Related Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S.J. Marx, M.D.	Chief, Min. Metab. Sec.	MDB, NIDDK
Others:	M.M. Bliziotes, M.D.	Medical Staff Fellow	MDB, NIDDK
	M. Nanes, M.D., Ph.D.	Medical Staff Fellow	MDB, NIDDK
	J. Barsony, M.D.	Guest Researcher	MDB, NIDDK
	M.L. Brandi, M.D.	Visiting Fellow	MDB, NIDDK
	G.D. Aurbach, M.D.	Chief	MDB, NIDDK
	U. Schumacher	Chemist	MDB, NIDDK

COOPERATING UNITS *(if any)*

Endocrine Section, USUHS, Bethesda
Biochemistry Department, University of Arizona, Tucson
Biochemistry Department, University of Wisconsin, Madison

LAB/BRANCH: Metabolic Diseases Branch

SECTION: Mineral Metabolism Section

INSTITUTE AND LOCATION: NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.00	1	2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The calciferols were the first class of hormonally active steroids to be discovered and also the first for which subjects with hormone resistance could be identified. With recognition that vitamin D is the precursor for 1,25-dihydroxyvitamin D, it has become possible to characterize defects in the activation (1-hydroxylation) of vitamin D and defects in the target action of activated (1,25-dihydroxy)vitamin D. We have demonstrated a broad spectrum of manifestations of hereditary resistance to $1,25(OH)_2D$ ranging from infantile rickets with alopecia and no intestinal response to calciferols to adult onset osteomalacia with satisfactory intestinal response to high doses of calciferols and with no epidermal abnormalities. A similar disorder has been recognized in new world monkeys. Cases with total lack of responses to calciferols have been treated with extroadinary doses of calcium administered intravenously. Cultured skin fibroblasts display many components of the $1,25(OH)_2D$ effector system. Skin fibroblasts from all subjects with hereditary resistance to $1,25(OH)_2D$ display abnormalities in this effector system, and defects in many discrete steps of this pathway have been identified with these cells. Other cells, such as bone cells, lymphocytes, and parathyroid cells can also be used to evalute actions of $1,25(OH)_2D$ in vitro.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 43009-01 MD

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*
Regulation of Mineral Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigetor.) (Name, title, leboretory, end institute effiliation)*

PI:	S.J. Marx, M.D.	Chief, Min. Metab. Sec.	MDB, NIDDK
Others:	M.M. Bliziotes, M.D.	Medical Staff Fellow	MDB, NIDDK
	M. Nanes, M.D., Ph.D.	Medical Staff Fellow	MDB, NIDDK
	M.L. Brandi, M.D.	Visiting Fellow	MDB, NIDDK
	U. Schumacher	Chemist	MDB, NIDDK
	G. Aurbach, M.D.	Chief	MDB, NIDDK

COOPERATING UNITS *(if eny)*

LAB/BRANCH
Metabolic Diseases Branch

SECTION
Mineral Metabolism Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Disorders of mineral metabolism have been evaluated with methods extending from epidemiology to cellular biology. Two forms of familial hyperparathyroidism have been characterized in detail. Familial hypocalciuric hypercalcemia is an autosomal dominant trait associated with abnormal interactions with calcium in parathyroid and kidney. Parathyroid cells from a presumed FHH homozygote showed a strikingly decreased sensitivity to secretory suppression by calcium. Familial multiple endocrine neoplasia type 1 is an autosomal dominant trait causing hyperfunction of parathyroids, pancreatic islet, and anterior pituitary. It is associated with gradual but abnormal proliferaltion of the tissues affected. Plasma from affected persons shows high mitogenic activity upon cultured bovine parathyroid cells.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43200-07 MD
(formerly Z01 AM 4300-06 MD)

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Disorders of Immune Regulation in Patients with Systemic Lupus Erythematosus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P. I.: G. C. Tsokos, Guest Researcher, MDB, NIDDK
J. E. Balow, Senior Investigator, MDB, NIDDK
G. Inghirami, Visiting Associate, MDB, NIDDK
P. L. Smith, Biologist, CC

COOPERATING UNITS *(if any)*

LAB/BRANCH

Metabolic Diseases Branch

SECTION

Kidney Disease Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1.75	.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Patients with systemic lupus erythematosus have been found to have various disturbances of the cell-mediated immune response. Cellular aberrations include enhanced spontaneous B cell activity with abnormal triggering in vitro, deficient immunoregulatory T cell circuits, deficient cytotoxic responses, including natural killer cell activity, alloantigen and viral cytotoxicity, and finally abnormal production of and response to different lymphokines. The goal of these studies is to further elucidate the mechanisms of these alterations of the immune system which are apparently involved in the pathogenesis of this disease. The modulation of the above disturbances by immunosuppressive agents, i.e. corticosteroids and cyclophosphamide, is actively studied, aiming at the restoration of normal immune status in these patients.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43201-02 MD
(formerly Z01 AM 43201-01 MD)

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Production and Characterization of Nephritic Factors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P. I.: G. C. Tsokos, Guest Researcher, MDB, NIDDK
M. Hiramatsu, Visiting Fellow, MDB, NIDDK
G. Thyphronitis, Visiting Fellow, MDB, NIDDK
J. E. Balow, Senior Investigator, MDB, NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Metabolic Diseases Branch

SECTION

Kidney Disease Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.25	1.25	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The nephritic factor of the alternative pathway of complement (NeFa) has been found in the sera of patients with membranoproliferative glomerulonephritis (MPGN) and partial lipodystrophy (PLD) and has been described as a factor which is able to induce cleavage of the third component of complement (C3) in normal human serum through the alternative pathway. It has been demonstrated that NeFa binds to and stabilizes C3bBb (alternative C3 convertase). NeFa appears to be antigenically and structurally similar to IgG and therefore it might be an autoantibody directed against C3bBb complex. Sera from patients with systemic lupus erythematosus (SLE) contain autoantibodies which bind and stabilize the C3 convertase of the classical pathway. This is classical pathway nephritic factor (NeFc). The relation between the development of renal lesions and the NeFa mediated persistent hypocomplementemia remains unexplained. To study the production of nephritic factors, we isolated B lymphocytes from peripheral blood mononuclear cells from patients with MPGN, SLE and normal individuals and established B cell lines by infecting them with Epstein-Barr virus (EBV) containing supernatants. We found that EBV transformed B cell lines derived from patients with MPGN, but not from normal individuals, produce an IgG molecule which stabilizes that C3bBb convertase activity. Supernatants from EBV transformed B cell lines from patients with SLE contain IgG molecules which stabilize C4b2a convertase activity. Full chemical and functional characterization of these antibodies to convertases is in progress.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43202-03 MD
(formerly Z01 AM 43202-02 MD)

PERIOD COVERED

October 1, 1986 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Human Immune Response by Complement

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P. I.: G. C. Tsokos, Guest Researcher, MDB, NIDDK
G. Thyphronitis, Visiting Fellow, MDB, NIDDK
J. E. Balow, Senior Investigator, MDB, NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Metabolic Diseases Branch

SECTION

Kidney Disease Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Complement factors and breakdown products block the differentiation of human B lymphocytes in vitro. Complement components and receptors also affect the generation of cytotoxic T cells in vitro. Understanding of the mechanism of regulation of immune responses by complement is crucial for the understanding of the immunopathogenesis of autoimmune diseases since they are frequently associated with complement activation and depression of complement factor levels.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43203-05 MD
(formerly Z01 AM 43203-04 MD)

PERIOD COVERED
October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Immunosuppression and Plasmapheresis in Goodpasture's Syndrome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P. I.: J. E. Balow, Senior Investigator, MDB, NIDDK
H. A. Austin, Expert, MDB, NIDDK

COOPERATING UNITS *(if any)* Foreign: None

Walter Reed Army Medical Center, Washington, D. C.
Scripps Clinic and Research Foundation, La Jolla, CA

LAB/BRANCH
Metabolic Diseases Branch

SECTION
Kidney Disease Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.25	.25	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Goodpasture's Syndrome is usually characterized by rapidly progressive glomerulonephritis due to autoantibodies against glomerular basement membrane (anti-GBM). This study will examine whether plasmapheresis significantly improves the results of standard immunosuppressive drug treatment of this disease. Patients will be randomly allocated to drug treatment alone or drugs plus plasmapheresis until anti-GBM levels are undetectable. Comparison will be made of the numbers of favorable outcomes and complication rates of these therapies at the end of 6 months of study. Analysis of the balance of efficacy, complications and expense of plasmapheresis in treatment of Goodpasture's syndrome will be facilitated by the results of this study.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43204-06 MD
(formerly Z01 AM 43204-06 MD)

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunosuppressive Drug Therapy in Lupus Glomerulonephritis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P. I.: J. E. Balow, Senior Investigator, MDB, NIDDK
J. H. Klippel, Senior Investigator, ARB, NIAMS
P. A. Plotz, Senior Investigator, ARB, NIAMS
A. D. Steinberg, Senior Investigator, ARB, NIAMS
R. L. Wilder, Senior Investigator, ARB, NIAMS
H. A. Austin, Expert, MDB, NIAMS

COOPERATING UNITS *(if any)*

Walter Reed Army Medical Center, Washington, D. C.
Foreign: None

LAB/BRANCH

Metabolic Diseases Branch

SECTION

Kidney Disease Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.2	1.4	0.8

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The efficacy of intensive, intermittent immunosuppressive drug therapy will be evaluated in patients with active lupus glomerulonephritis over a 30 month study period. Patients with renal biopsy documented active glomerulonephritis with or without renal functional deterioration will be treated with low dose corticosteroids and randomized to receive (a) intravenous pulse methylprednisolone monthly for 6 months or (b) intravenous pulse cyclophosphamide monthly for 6 months or (c) intravenous pulse cyclophosphamide monthly for 6 months and then every 3 months for the remaining 24 months of the study. During the final 24 months of the study, all patients will receive low dose prednisone. Active disease, as manifested by renal functional deterioration, increased proteinuria or worsened urinary sediment, will be treated by increased prednisone. Comparison will be made of the number of favorable outcomes of renal function, glomerular pathology and drug related toxicities achieved by each treatment group at the end of the 6th and 30th study months. Between April 1981 and July 1986 there have been more than 60 patients entered into this protocol.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

(formerly

PROJECT NUMBER

Z01 DK 43205-09 MD
Z01 AM 43205-08 MD)

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Renal Biopsy Pathology in Systemic Lupus Erythematosus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P. I.: J. E. Balow, Senior Investigator, MDB, NIDDK
H. A. Austin, Expert, MDB, NIDDK
D. E. Webb, Expert, CC

COOPERATING UNITS *(if any)*

Armed Forces Institute of Pathology, Washington, D. C.

Foreign: None

LAB/BRANCH

Metabolic Diseases Branch

SECTION

Kidney Disease Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.25	.25	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The pathogenetic mechanisms underlying the different forms of lupus nephritis are being investigated. Detailed analysis of renal biopsy pathology is being conducted on specimens from patients with systemic lupus erythematosus. Biopsies are classified by major category of lupus nephritis, as well as scored on a semiquantitative scale for specific histologic changes indicating the degree of activity and of chronic sclerosing features. The patterns of immune complex deposition and lymphoid cell interaction with different segments of the nephron are being investigated by immunohistologic techniques and electron microscopy. These approaches have facilitated the analysis of the effects of various types of immunosuppressive agents used to halt the progression of lupus nephritis and they will enhance our understanding of the pathogenesis of this diseases.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43206-02 MD
(formerly Z01 AM 43206-01 MD)

PERIOD COVERED
October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunoregulatory Disorders in Patients With Juvenile Rheumatoid Arthritis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P. I.: G. C. Tsokos, Guest Researcher, MDB, NIDDK
S. R. Pillemer, Medical Staff Fellow, MDB, NIDDK

COOPERATING UNITS *(if any)*

LaRabida Children's Hospital, Chicago, IL (Dr. D. Magilavy)
Foreign: None

LAB/BRANCH
Metabolic Diseases Branch

SECTION
Kidney Disease Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.25	.25	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Juvenile rheumatoid arthritis (JRA) is the most frequent pediatric connective tissue disease. It is still unclear if the disease represents a single entity or several diseases with multiple pathogenetic mechanisms. Among the possible causes are infection, trauma, stress, immunogenetic predisposition and autoimmunity. The autoimmune nature of the disease has been supported by: 1) increased frequency of antinuclear antibodies and rheumatoid factor in the sera of these patients, 2) the presence of circulating autoantibodies directed against the T cell subpopulation which induces the generation of suppressor cells, 3) segregation of certain HLA antigens such as HLA-DW5 and HLA DW-8, and 4) sporadic, not well-documented reports of deficient peripheral immune cell functions. Careful analysis of various cellular immunological functions in vitro using modern more sophisticated immunological techniques is absolutely needed in order to shed light on the immune status of patients with JRA.

Major findings to date include: increased numbers of pre-activated B and T lymphocytes in the peripheral blood and altered functions of selected T cell helper and suppressor cell assays. Studies are in progress to identify the pathogenesis of these immunoregulatory disturbances and to identify potential factors which can restore immune function toward normal in JRA patients.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43207-02 MD
(formerly Z01 AM 43207-01 MD)

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Viral Interferon and Localization of Pre-formed Complexes in Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P. I.: G. Striker, Director, DKUHD, NIDDK
L. Striker, Expert, MDB, NIDDK

COOPERATING UNITS *(if any)*

Foreign: None

Department of Medicine, University of Washington School of Medicine, University of Washington, Seattle, Washington (Dr. M. Mannick).

LAB/BRANCH

Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Newborn mice of both sexes were injected from day 1 to 7 with 1 million international units of viral interferon provided by Dr. Gresser of Villejuif, Paris, France. Seven days after the last injection, 5 mg/kg of pre-formed antigen antibody complexes in 5 times antigen excess were injected intravenously. Serial blood samples were drawn as described in previous publications and the animals were sacrificed serially. Light, immunofluorescence and electron microscopic studies were performed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 43208-02 MD

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Differentiation and Matrix

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

L. Striker, Expert, MDB, NIDDK
G. Striker, Director, DKUHD, NIDDK
K. MacKay, Medical Staff Fellow, MDB, NIDDK

COOPERATING UNITS *(if any)*

Laboratory of Developmental Biology and Anomalies, NIDR (Drs. G. Martin and H. Kleinman).
Foreign: None

LAB/BRANCH

Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0		

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 43209-02 MD

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Polycystic Kidney Disease Studies In Vitro

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

L. Striker, Expert, MDB, NIDDK
G. Striker, Director, DKUHD, NIDDK
E. J. Blanchette-Mackie, Research Biologist, LCDB, NIDDK

COOPERATING UNITS *(if any)*

Department of Medicine, University of Colorado, Denver, Colorado (Dr. P. Wilson).
Foreign: None

LAB/BRANCH

Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0		

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43210-02 MD
(formerly Z01 AM 43210-01 MD)

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Glomerular Disease in Transgenic Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P. I.: K. MacKay, Medical Staff Fellow, MDB, NIDDK
G. Striker, Director, DKUHD, NIDDK
L. Striker, Expert, MDB, NIDDK
S. Elliot, Bio. Lab. Tech., MDB, NIDDK

COOPERATING UNITS *(if any)*

Foreign: None

School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA (Drs. R. Brinster and C. Pinkert).

LAB/BRANCH

Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	2	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have identified several lines of mice transgenic for the early region of simian virus 40 (SV40) that develop renal abnormalities. While the specific pattern of pathology differs between different SV40 DNA constructs and different insertion sites a large number of the animals develop progressive glomerulosclerosis. As there are no evident extrarenal sources of injury, and because expression of the foreign DNA (T antigen) has been documented to occur in the whole kidney we suspect that the glomerular disease may be secondary to abnormalities in glomerular cell function induced by T antigen.

We have identified one line of mice whose glomerular abnormalities closely resemble those seen in human focal glomerulosclerosis and plan to evaluate it in detail. We have examined kidneys from these mice using light, electron and immunofluorescence microscopy. We plan to correlate the temporal development of the glomerular lesions with expression of T antigen, and to search in vivo for abnormalities in glomerular cell behavior which would be expected to occur in a T antigen expressing cell, specifically, increased proliferation. It is hoped that evaluation of this new model of glomerulosclerosis when combined with the in vitro studies described in a separate proposal will lead to a greater understanding of the cellular and molecular abnormalities of glomerular cells which may contribute to the development of glomerulosclerosis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43211-02 MD
(formerly Z01 AM 43211-01 MD)

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Histopathology of Renal Lesions in Pima Indians

PRINCIPAL INVESTIGATOR *(List other professional personnal below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P. I.: L. Striker, Expert, MDB, NIDDK
G. Striker, Director, DKUHD, NIDDK
F. Conti, Visiting Fellow, MDB, NIDDK

COOPERATING UNITS *(if any)*

Epidemiology and Clinical Research Branch, NIDDK, Phoenix, Arizona (Dr. P. Bennett).
Foreign: None

LAB/BRANCH

Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.5	.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Autopsies from diabetic and non-diabetic Pima Indians will be examined from a series drawn as a representative sample of the autopsy population by Dr. Peter Bennett. Routine light microscopic studies, and potentially electron microscopic studies, will be performed to assess the histopathologic lesions present in these autopsy specimens. Particular attention will be paid to epithelial basement membranes and vascular extracellular matrix areas.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43212-02 MD
(formerly Z01 AM 43212-01 MD)

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Coagulation Studies Using Human Glomerular Endothelial Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Neme, title, laboratory, and institute affiliation)*

P. I.: M. Lange, Guest Researcher, MDB, NIDDK
L. Striker, Expert, MDB, NIDDK
G. Striker, Director, DKUHD, NIDDK

COOPERATING UNITS *(if any)*

University of Michigan, Ann Arbor, Michigan (Dr. R. Wiggins).

Foreign: None

LAB/BRANCH

Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	2	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use stenderd unreduced type. Do not exceed the space provided.)*

Human glomerular endothelial cells are isolated and cloned from glomeruli obtained from nephrectomy specimens which have been removed for medical or surgical reasons. Some glomeruli will be obtained from specimens which were initially designated to be used as cadavor transplants but were not able to be utilized for technical or other reasons. The principal assays to be used will be to assess the procoagulant activity of supernatants and cytoplasmic preparations from the cells.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 AM 43213-02 MD

PERIOD COVERED
October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Isolation and Characterization of Voluntary Muscle Cells In Vitro

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

L. Striker, Expert, MDB, NIDDK
G. Striker, Director, DKUHD, NIDDK
F. Miller, Medical Staff Fellow, ARB, NIAMS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0		

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43214-02 MD
(formerly Z01 AM 43214-01 MD)

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cell and Molecular Biology of Glomerular Cells Derived From Transgenic Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P. I.: K. MacKay, Medical Staff Fellow, MDB, NIDDK
L. Striker, Expert, MDB, NIDDK
G. Striker, Director, DKUHD, NIDDK
S. Elliot, Bio. Lab. Tech., MDB, NIDDK

COOPERATING UNITS *(if any)*

School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania (Drs. R. Brinster and C. Pinkert).
Foreign: None

LAB/BRANCH

Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.25	1.25	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Current models of glomerulosclerosis (GS) have yielded little information about the cellular and molecular abnormalities that are critical in the initiation and progression of this disease. The complexity of the kidney and glomerulus make isolation and examination of pure cultured populations of glomerular cells an attractive method for beginning to answer these questions. Unfortunately other models of GS involve extrarenal causes of glomerular injury. Because of this it is quite likely that glomerular cells isolated from these models would not maintain the abnormal behavior in vitro which led to the development of GS in vivo.

We have identified several lines of mice transgenic for the early region of simian virus 40 (SV40) that develop progressive glomerulosclerosis. As there are no evident extrarenal sources of injury, and since expression of the foreign DNA has been documented to occur in whole kidney we suspect that the glomerular disease may be secondary to expression of the foreign DNA by glomerular cells in vivo.

We have isolated lines of glomerular endothelial, mesangial, and epithelial cells from transgenic mice and have isolated pure cultures of mesangial and epithelial cells from their normal litter mates. As preliminary data from the in vivo model indicates that proliferation of glomerular cells is an early event in the development of GS in transgenic mice we plan to begin the evaluation of these

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 43215-02 MD

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Effect of Endotoxin on Human Glomerular Endothelial Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

L. Striker, Expert, MDB, NIDDK
G. Striker, Director, DKUHD, NIDDK

COOPERATING UNITS *(if any)*

Department of Medicine, University of Washington, Seattle, Washington (Drs. G. Raghu and J. Harlan).
Foreign: None

LAB/BRANCH

Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0		

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is inactive.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43216-02 MD
(formerly Z01 AM 43216-01 MD)

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Progression of Glomerulosclerosis in Experimental Membranous Glomerulonephritis

PRINCIPAL INVESTIGATOR *(List other professional personnal below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P. I.: L. Striker, Expert, MDB, NIDDK
G. Striker, Director, DKUHD, NIDDK

COOPERATING UNITS *(if any)*

Department of Medicine, University of Washington, Seattle, Washington (Drs. S. Adler and W. G. Couser).
Foreign: None

LAB/BRANCH

Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.5	.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Sprague-Dawley rats are uninephrectomzied and are then immunized with a sheep antibody directed against brush border antigen. This results in a membranous glomerulonephritis. This study is designed to study how glomerular sclerosis progresses. Immunofluorescence studies allow us to study the topography and composition of extracellular matrix. Preliminary studies show that immune deposits disappear within 36 weeks whereas, glomerular sclerosis progresses. Extracellular material appears to contain collagen type IV.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 43217-02 MD

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Renal Lesions in Leukemias, Lymphomas and Carcinomas

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

L. Striker, Expert, MDB, NIDDK
G. Striker, Director, DKUHD, NIDDK

COOPERATING UNITS *(if any)*

Hopital Tenon, Paris, France (Dr. F. Mignon); Pitt County Memorial Hospital, Greenville, North Carolina (Dr. D. Dabbs).

LAB/BRANCH

Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0		

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is inactive.

PHS 6040 (Rev 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 43218-01 MD

PERIOD COVERED
October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Development of Human Glomerular Cell Lines

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P. I.: M. A. Lange, Guest Researcher, MDB, NIDDK
G. Striker, Director, DKUHD, NIDDK
L. Striker, Expert, MDB, NIDDK
S. Elliot, Bio. Lab. Tech., MDB, NIDDK

COOPERATING UNITS *(if any)*

Synergen, Colorado (K. Van Doren).
Foreign: None

LAB/BRANCH
Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	2	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Primary outgrowth of human glomeruli containing mixed populations of epithelial cells, mesangial cells, and endothelial cells were infected with a recombinant adenovirus 5-simian virus 40. Foci of transfected cells arose which are being isolated and characterized. These cell lines all exhibit nuclear staining for the SV40 large T antigen. After multiple passage, their morphologic characteristics are similar to their non-transfected counterparts by immunofluorescence. Further phenotypic properties are being explored. Establishment of these lines will allow sufficient numbers of cells for study of human glomerular cell function.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43219-01 MD

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Glomerular Endothelial Cells and Immune Complexes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P. I.: M. A. Lange, Guest Researcher, MDB, NIDDK
L. Striker, Expert, MDB, NIDDK
G. Striker, Director, DKUHD, NIDDK
L. Agodoa, Medical Officer, MDB, NIDDK
S. Elliot, Bio. Lab. Tech., MDB, NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Very little is known about the initial subendothelial localization of immune complexes in glomeruli. Using human glomerular endothelial cell lines established in this laboratory, cells will be evaluated for transcytosis of immune complexes of human serum albumin and anti-human serum albumin (HSA). In order to determine whether immune complex interaction with glomerular endothelial cells is an active or passive process, three methods will be employed. First, using radiolabeled immune complexes dose response curves will be obtained testing for the presence of saturation kinetics. Second, endothelial cell-immune complex interaction will be followed using video enhanced fluorescence microscopy. Third, complexes and endothelial cells will be evaluated by immunoelectron microscopy.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 43220-01 MD

PERIOD COVERED
October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regulation of Expression of Angiotensin Converting Enzyme in Renal Glomeruli

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P. I.: K. Bernstein, Special Assistant to Associate Director, DKUHD

COOPERATING UNITS *(if any)*

LAB/BRANCH
Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.5	.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

It has been suggested on an experimental basis that elevated intraglomerular pressure leads to glomerulosclerosis. The regulation of angiotensin converting enzyme (ACE) production plays a central role in maintaining normal intraglomerular pressure. This project is designed to isolate the gene encoding ACE from mouse kidney to further study the regulation and expression of the enzyme.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 43221-01 MD

PERIOD COVERED
October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biology of Insulin Receptors in Glomerular Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigetor.) (Name, title, leboratory, and institute effiliation)*

P. I.: F. Conti, Visiting Fellow, MDB, NIDDK
L. Striker, Expert, MDB, NIDDK
G. E. Striker, Director, DKUHD, NIDDK
K. MacKay, Medical Staff Fellow, MDB, NIDDK
M. A. Lange, Guest Researcher, MDB, NIDDK
S. Elliot, Bio. Lab. Tech., MDB, NIDDK

COOPERATING UNITS *(if any)*

Diabetes Branch, NIDDK (M. Lesniak)

Foreign: None

LAB/BRANCH
Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	3	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We propose to study insulin specific binding on glomeruli from mice and humans. Binding of insulin to mesangial cells from normal transgenic mice, and human kidneys is being investigated. The nature of the receptor will be studied and elucidated.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 43222-01 MD

PERIOD COVERED
October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Pathogenesis of Murine Lupus Nephritis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P. I.: H. A. Austin, Expert, MDB, NIDDK
J. E. Balow, Senior Investigator, MDB, NIDDK

COOPERATING UNITS *(if any)*
Armed Forces Institute of Pathology, Washington, D. C. (Drs. Antonovych and Sabnis).
Foreign: None

LAB/BRANCH
Metabolic Diseases Branch

SECTION
Kidney Disease Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The pathogenetic mechanisms underlying the diverse forms of murine lupus nephritis are being investigated. Renal morphology is being studied by light, immunofluorescence, immunoperoxidase and electron microscopy to delineate the types of glomerular and tubulo-interstitial lesions, as well as the characteristics of the immune deposits and the lymphoid cell proliferation. The impact of provocative maneuvers on serologic and renal histologic features is being examined to develop a model of a flare of lupus nephritis.

PHS 6040 (Rev. 1/94) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 43223-01 MD

PERIOD COVERED
October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Crescentic glomerulonephritis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P. I.: J. E. Balow, Senior Investigator, MDB, NIDDK

H. A. Austin, Expert, MDB, NIDDK
D. E. Webb, Expert, CC

COOPERATING UNITS *(if any)*

LAB/BRANCH
Metabolic Diseases Branch

SECTION
Kidney Disease Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Crescentic glomerulonephritis is a rapidly progressive renal disease with a high risk of development of end-stage renal failure within a few weeks or months of onset. The choice and effectiveness of therapy are controversial. High-dose pulse methylprednisolone is widely preferred for treatment of crescentic glomerulonephritis at the present time, but its efficacy is acknowledged to be less than ideal in preserving renal function. Our study is designed to test the efficacy of intensive, intermittent immunosuppressive drug therapy in patients with crescentic glomerulonephritis over a 6 month study period. Patients with renal biopsy documented active crescentic glomerulonephritis will be treated with a short course of oral corticosteroids and randomized to receive in addition: (a) intravenous methylprednisolone monthly for 6 months, or (b) intravenous cyclophosphamide monthly for 6 months. Comparisons will be made of the number of favorable outcomes of renal function and renal pathology, as well as drug-related toxicities for each treatment group.

PHS 6040 (Rev. 1/84) GPO 914-918

Annual Report of the Clinical Endocrinology Branch, National Institute of Diabetes and Digestive and Kidney Diseases

Dr. Harold Edelhoch, an illustrious member of CEB for thirty years, died after a brief illness on January 15, 1986. After joining CEB, Dr. Edelhoch's extensive work on the chemistry of thyroglobulin established him as the world's foremost authority on this subject. The recent work of his laboratory on the chemistry of clathrin has been carried forward by his coworkers.

The Branch has continued to be host to a number of scientists from abroad. During the past year, they included scientists from Brazil, Greece, India, Italy and Japan. Collaboration with former NIH visiting scien- tists in Pisa and Rome, Italy, has been active.

The Branch has been host to two Fogarty Scholars during 1985-6. Dr. Jamshed Tata, from the National Institute for Medical Research, Mill Hill, England, and Dr. Teruo Matsuura from Kyoto University, Japan. Dr. Tata has collaborated with Drs. Jacob Robbins and Alfredo Pontecorvi on the differentiation of skeletal muscle cells as influenced by insulin. Dr. Matsuura has worked with Dr. Hans Cahnmann on problems in photochemistry. Both have contributed significantly to the activities of the Branch.

I. Thyroid Biochemistry and Pathophysiology

A. Thyroxine-Protein Interactions

While TBG, prealbumin and albumin constitute the major thyroid hormone binding system in human plasma, convincing evidence for similar binding to plasma lipoproteins is lacking. Such binding, although expected to be in relatively small amount, could have a significant role in hormone transport to certain tissues. The initial approach was to confirm that T_4 and T_3 interact with lipoproteins and, if so, to determine whether they are associated with the lipids or the apoproteins. The technique of Sepharose CL-6B chromatography was used to measure ^{131}I-labeled hormone binding in plasma from normal subjects or patients with congenital lipoprotein abnormalities. The studies showed unequivocally that hormone is bound to each of the lipoprotein classes (VLDL, LDL and HDL) and a stereospecific interaction with certain of the apoproteins was demonstrated. The major portion of lipoprotein-bound hormone is associated with the high density lipoprotein (HDL) and is, therefore, proportional to apoprotein content rather than lipid content. Altogether, only 1 to 2 percent of plasma thyroid hormone is transported by lipoproteins. Future studies will explore whether lipoproteins that bind specifically to certain tissues for delivery of lipid molecules such as cholesterol can simultaneously transfer thyroid hormone to the cells. (Robbins, Benvenga)

A commonly used commercial assay kit for measuring thyroxine-binding globulin (TBG) in plasma has been found to give erroneous low results in patients with nonthyroid illness (NTI). The mechanism of this anomaly was investigated by studying the effect of fatty acids, known to be

elevated in NTI, as well as the influence of certain drugs. It was found that unsaturated fatty acids and drugs such as the diuretic, furosemide, and the antiinflammatory analgesics, diclofenac and mefenamic acid, are effective inhibitors of thyroxine-binding to TBG, thus accounting for the measurement error. It was concluded that conventional radioimmunoassay is a more reliable method for measuring TBG, but that the immunoradiometric assay employing radioactive thyroxine can be used as a convenient and rapid method to test for thyroid hormone binding inhibitors. (Robbins, Benvenga)

Cultured human hepatoma cells were previously used to study the biosynthesis of TBG. This system was used to investigate the reported specific depression of plasma TBG induced by the antineoplastic drug, L-asparaginase. The drug was shown to inhibit the synthesis of TBG, but the effect was not specific since albumin synthesis was also reduced. (Robbins, Bartalena)

B. Thyroid Hormone Metabolism

Recent studies have revealed that cells take up thyroid hormone by a stereospecific, energy dependent mechanism in addition to passive diffusion. This process has been investigated in skeletal muscle, a tissue that differs from most others in that triiodothyronine (T_3), the active hormone at the nuclear level, is derived almost exclusively from the plasma rather than from intracellular deiiodination of thyroxine (T_4). In the intact soleus muscle, a physiological concentration of insulin was shown to increase the specific uptake, thus suggesting a regulatory mechanism at this level of hormone metabolism. To understand the mechanism of the insulin action, the role of cation transport at the plasma membrane was investigated. It was shown that specific T_3 uptake is dependent on sodium ion transport and can be increased by monensin, a drug that increases the rate of Na^+ entry. When lithium ion was used to replace Na^+, the inhibitory effect could be abolished by amiloride, a specific inhibitor of the Na^+/H^+ exchanger. Ouabain, an inhibitor of Na^+/K^+ exchange, had a less complete inhibitory effect on T_3 transport. Although not easy to understand, the results indicate that sodium transport systems in the plasma membrane are involved in T_3 transport into muscle. Furthermore, the finding that T_4 uptake was not affected confirmed previous indications that muscle cells do not accumulate T_4 by a regulated, specific mechanism. (Robbins, Centanni, Pontecorvi)

Cultured rat muscle cells, a line capable of undergoing differentiation from myoblasts to mature myocytes, were used to elucidate the influence of specific T_3 uptake on hormone accumulation in the cell nucleus. Inhibitors of T_3 transport with different mechanism of action - energy metabolism inhibitors such as antimycin, endocytosis inhibitors such as monodansyl cadaverine, and cytoskeleton destabilizers such as cytocholasin - all prevented nuclear T_3 uptake. These experiments were performed by incubating intact myoblasts with labeled hormone and then measuring its distribution after cell disruption in the cytosol and nuclear fractions. Contrary to recent work with hepatocytes, the results showed that specific T_3 uptake in myoblasts occurs only at the plasma membrane and that regulation of hormone entry into muscle cells is exerted at this level. (Robbins, Pontecorvi, Lakshmanan, Phyillaier)

C. Thyroid Hormone Action

It is currently believed that thyroid hormone increases protein synthesis by activating sensitive genes through a specific T_3 receptor. Malic enzyme, a T_3-sensitive protein in rat liver, has been under study as a model for this type of thyroid hormone action. Previous work demonstrated that the increase in malic enzyme mRNA (ME mRNA) caused by T_3 is only partially due to increased gene transcription, the remainder resulting from stabilization of nuclear ME mRNA. It has now been shown that increased carbohydrate intake stimulates malic enzyme synthesis by a different mechanism, acting only to stabilize cytoplasmic ME mRNA. These findings account for the additive effects of carbohydrate and thyroid hormone on malic enzyme synthesis, which is increased 4-fold by carbohydrate in hypothyroidism and 12- to 15-fold in hyperthyroidism. Furthermore, carbohydrate acts only on liver whereas thyroid hormone also increases ME synthesis in the heart, but not in brain. Thus the action of thyroid hormone on malic enzyme synthesis is complex, involving tissue-specific gene transcription and mRNA stabilization. (Nikodem, Dozin, Rall)

In order to define the mechanism by which T_3 increases ME gene transcription, the structure of the gene and its interaction with the T_3 receptor protein needs to be clarified. ME cDNA, the structure of which had been completely analyzed in previous work, have been used to screen a rat liver genomic library. Thus far, 10 exons and 12 introns have been mapped, and only two gaps exist: the second exon and 423 nucleotides at the position of the seventh and eighth exons. The length of the partially mapped ME gene exceeds 84 kilobases of DNA. One of the genomic clones, obtained by use of a cDNA fragment encompassing the 5' end, contained the 5'-flanking region, and about 900 nucleotides of this region have been sequenced. None of the expected concensus sequences (TATA, CAAT) characteristic of eukaryotic promoters were found. Instead, this region is G/C rich and contains, in direct repeat, six CCGCCC hexanucleotides upstream from the transcription initiation site. Five of these lie between -36 and -363, one is in the untranslated mRNA region, and one is at the beginning of the first exon and in the opposite orientation. Deletion analysis is used to characterize this apparent promoter region of the malic enzyme gene. Deleted gene fragments have been inserted into the bacterial chloramphenicol acetyl transferase (CAT) gene and transfected into several cell types (monkey kidney, Chinese hamster ovary, pituitary GH1, rat hepatoma H-35). Preliminary results show that -363 base pairs of the ME gene 5' region can direct expression of the CAT gene whereas -40 and -83 base pairs cannot, and that this expression occurs only in Chinese hamster ovary cells. (Nikodem, Morioka, Rall)

Further characterization of the T_3 receptor protein has been accomplished by refinement of the photoaffinity labeling system. It is now clear that the receptor in liver nuclei is a 57 kDa protein, and that it is also abundant in heart and brain but not in testis or spleen, tissues that do not respond to thyroid hormone. Preliminary studies on the interaction of the receptor with DNA have employed calf thymus DNA-cellulose and have shown that the 57 kDa component in receptor preparations is strongly absorbed. It is now planned to study receptor interaction with the 5'-flanking region of the malic enzyme gene. (Nikodem, Cahnmann, Sheer)

The phenomenon of relative DNAase sensitivity of genomic DNA sequences has become a popular tool to analyze chromatin structure in relation to hormonal regulation of gene expression. This technique has been applied to the malic enzyme gene and its regulation by thyroid hormone. Approximately -1.4 kb to +1.0 kb of genomic DNA, encompassing the 5' end of the gene and part of its 5'-flanking region has been examined. Although hyperthyroid liver chromatin has a 5' site that is more sensitive to DNAase than in hypothyroid liver, this has not been demonstrated in the malic enzyme gene. The 1 kb region immediately upstream from the start of transcription has three GGCC sequences, but these are methylated, in contrast to the typically non-methylated GGCC sequences found at hypersensitivity sites. Hypersensitivity sites have also been sought in the 3' downstream region of the ME gene, constituting the polyadenylation site. Multiple hypersensitivity sites have been found in a 4.4 kb downstream region, and two relatively strong sites have been partially mapped. However, they are present in hypothyroid as well as in hyperthyroid liver. Since it is very unusual that an active gene should not possess sites that are hypersensitive to DNAase, the possible existence of heterogeneity of malic enzyme gene activity is being explored by the technique recently proposed by Harold Weintraub. If such heterogeneity is found, it would indicate that thyroid hormone regulation in liver includes "cell recruitment" as well as the already demonstrated transcription initiation and RNA stabilization. (Nikodem, Usala)

It was reported previously that thyroid hormone caused an increase in an mRNA (4-12B) in rat liver having extensive nucleotide sequence homology with mouse contrapsin, a trypsin inhibitor. The amino acid sequence homology was 66.8 percent. It has now been shown that T_3 increases the transcription rate 4-fold in the liver of hypothyroid rats, whereas the mRNA level is increased 12-fold. As in the regulation of malic enzyme synthesis, there is a large, post-translational effect of thyroid hormone, indicating that both 4-12B and malic enzyme mRNA belong to a class of messenger RNA that is stabilized by thyroid hormone. (Nikodem, Tecce, Dozin, Magnuson)

In related work, a study of creatine kinase (CK) activity in skeletal muscle cells (L6A1R5) was initiated. These cells undergo differentiation in response to insulin, during which there is a 203-fold increase in enzyme activity. In contrast, CK mRNA increased only 17.5-fold. This indicates that insulin also has a complex effect which includes an increase in the rat of protein synthesis or processing in addition to the increase in mRNA level. A possible effect of insulin on mRNA stabilization is currently being examined. (Pontecorvi, Nikodem, Tata, Robbins, Phyillaier)

D. Studies in Thyroid Disease

Additional patients have been recruited into the study of the use of lithium carbonate in therapy of metastatic thyroid cancer with radioactive iodine. This agent has the unique effect of slowing iodine release without altering iodine uptake. To date, 14 studies in 10 patients have been completed. It has been shown that a beneficial effect is to be expected only when the rate of iodine secretion from the tumor is rapid

relative to the rate of isotopic decay of ^{131}I (8 days). In selected patients, lithium can improve the tumor to whole body radiation ratio, and this is of benefit in therapy. Further experience is required to define the place of this adjuvant to ^{131}I therapy. (Robbins, Movius, Lakshmanan)

II. Mechanism of Cell Secretion

In many types of cells, secretion following hormonal stimulation is mediated through the adenyl cyclase system and increased cAMP production. This does not appear to be the case in adrenal cells. In addition to studies with Y-1 mouse adrenal tumor cells, the wild type, a variant cell line (kin-8) has been employed which is deficient in C-AMP dependent protein kinase. Unlike some other cells, Y-1 cells do not increase their cAMP output upon exposure to antimitotic drugs such as colchicine, vinblastine or podophyllotoxin, which readily increase steroidogenesis. Stabilization of tubulin by taxol or vinblastine blocks steroidogenesis induction by ACTH or cholera toxin without major effects on cAMP levels. In kin-8 cells, colchicine and podophyllotoxin stimulate steroidogenesis to the same degree as in Y-1 cells, although the absolute yield of steroids is lower in the kinase deficient cells. It is suggested that antimitotic agents stimulate adrenal steroidogenesis by a cAMP-independent pathway by facilitating cholesterol access to the mitochondrial site of side chain cleavage. It is evident that, in adrenal cells, cAMP stimulation is not an obligatory step in steroidogenesis. (Wolff, Sackett, Knipling)

Based on the results of limited proteolytic cleavage of rat brain tubulin, a model for the substructure of the tubulin dimer, and the orientation of the dimer in the polymer, has been proposed that accommodates internal cleavage in the dimer but not in the polymer and access to the C-termini in both forms. Both the α and ß subunits each have a single, highly reactive site for a variety of proteases, but the cleavage site differs in the two subunits. In the α subunit, trypsin cleavage occurs after residue 339, and in the ß subunit, chymotrypsin cleavage occurs after residue 281, resulting in two unequal fragments, 38 and 14 kDa in α and 34 and 21 kDa in ß. Following cleavage, the two pieces of each subunit remain associated. When the tubulin dimer is assembled into microtubules or sheets, the internal site is protected against proteolytic cleavage whereas the carboxy terminal, subtilisin-sensitive sites remain exposed. These changes have major effects on polymerization of tubulin; carboxy terminus cleavage promotes assembly whereas internal cleavage inhibits assembly. As a result of differential rates of C-terminal cleavage of α and ß subunits, subtilisin treatment can lead to mixed polymers of differing composition. These polymers can form at concentrations of tubulin that are incapable of polymerizing from native tubulin and even a few percent of tubulin S can promote copolymerization below the usual critical concentration. This suggests a possible role for *in vivo* proteolysis in the induction of microtubule formation. (Wolff, Sackett)

III. Adenylate Cyclase of Bacterial Origin

Further clarification of the stimulation of *Bordetella pertussis* adenylate cyclase by calmodulin has been obtained through improvements

in the purification of calmodulin proteolytic fragments. This bacterial cyclase is an important element in Bordetella toxicity, and its response has been compared to that of Ca^{2+}/calmodulin dependent cyclic nucleotide phosphodiesterase of bovine brain. Highly purified calmodulin fragments 1-77, 1-90, 78-148 and 107-148 all stimulated the bacterial cyclase but were devoid of phosphodiesterase-stimulating activity. Stimulation occurred in the absence of Ca^{2+}, but was enhanced in the presence of calcium, and it was concluded that the two C-terminal Ca^{2+} binding sites of calmodulin accounted for most of the enhancement. (Wolff, Knipling)

IV. Interactions of Proteins with Cell Membranes

Structural and biological properties of clathrin, the major protein of the coated pits on the plasma membrane of cells, have been further elucidated. A clathrin-associated protein having a molecular weight near 100,000 has been purified from bovine brain, using coated vesicles as starting material. The precise molecular weight, 114,000, and its tertiary structure were determined. Addition of the "100 kDa" protein to clathrin at a mole ratio of one results in polymerization to uniform sized clathrin baskets of sedimentation velocity 150 S, and the rate of clathrin polymerization is markedly enhanced. It was also shown that the 8 S clathrin molecule forms a homogeneous species with a sedimentation coefficient of 24 S under low salt conditions. Increasing the salt concentration converts all the 27 S, species into 150 S baskets. The 27 S species has a molecular weight six times that of the clathrin protomer and is the result of a highly cooperative reversible self association of the protomer. Trypsin digestion of the baskets removes about one-third of the mass from the distal portions of the arms of the clathrin triskelion without affecting the clathrin-basket transitions. (Edelhoch, Prasad, Lippoldt, Yano)

A biosynthetic study of rat liver coated vesicle proteins was undertaken using in vivo labeling with [^{35}S]methionine. Labeling of the clathrin heavy chain (180 kDa) and a 90 kDa clathrin-associated protein was detected within 5-1/2 minutes, increased rapidly during the next 2 hours and then more slowly between 4 and 16 hours. These two proteins accounted for 75 percent of the labeling in all coated vesicle proteins. Two other proteins, 53 and 68 kDa, decreased with time. The evidence suggested that clathrin heavy chains might be recycled during coated vesicle formation and that heterogeneity with respect to protein composition might exist. (Edelhoch, Pierce)

Coated vesicles from bovine thyroid glands were isolated and shown to resemble those from other organs. Thyroglobulin (Tg) was found to be associated with purified coated vesicles. Most of this Tg, however, could be removed by dissociation of the clathrin coat and the remainder was removed from the smooth vesicles by trypsin digestion, indicating that Tg was on the external surface, oriented toward the interior of the cell. No evidence for Tg-receptor complexes within the vesicles was found. Labeling of Tg with [^{35}S]methionine in thyroid slices showed no association of Tg with coated vesicles. It was concluded that coated vesicles are not involved in the secretion of newly synthesized Tg from the cell to the follicle lumen and do not appear to participate either in exocytosis or endocytosis of Tg. (Edelhoch, Pierce)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 45000-19 CEB
Formerly Z01 AM 45000

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Thyroxine-Protein Interactions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. Robbins	Chief	CEB, NIDDK
Others:	S. Benvenga	Guest Researcher	CEB, NIDDK
	R. E. Gregg	Senior Investigator	IR, NHLBI
	H. J. Cahnmann	Scientist Emeritus	CEB, NIDDK
	H. Edelhoch	Chief, Protein Structure Section	CEB, NIDDK

COOPERATING UNITS *(if any)*

University of Rome, Italy (S. Grimaldi); University of Pisa, Italy (L. Bartalena); University of Messina, Italy (S. Benvenga)

LAB/BRANCH

Clinical Endocrinology Branch

SECTION

Hormone Metabolism and Action Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	.8	.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

While TBG, prealbumin and albumin constitute the universally accepted major thyroid hormone binding system in human plasma, convincing evidence for similar binding to plasma lipoproteins is lacking. In a systematic study using Sepharose CL-6B chromatography to examine plasma from normal subjects and patients with genetic lipoprotein abnormalities, the interaction between thyroid hormone and the individual lipoprotein classes (VLDL, LDL and HDL) was unequivocally demonstrated. Binding of thyroxine was greater than that of triiodothyronine, and binding appeared to depend on the protein moieties of the ipoproteins. Whether this interaction, which accounts for less than 1-2% of thyroid hormone transport, plays any physiological role requires further study.

Since serum TBG levels measured by an immunoradiometric assay (IRM, Corning kit) in patients with nonthyroid illness (NTI) are lower than those measured by radioimmunoassay (RIA), the effect of lipids known to be elevated in NTI, and various drugs, were tested for their ability to inhibit the binding of thyroxine to TBG. Unsaturated fatty acids and drugs such as furosemide, diclofenac and mefenamic acid were effective inhibitors. It was concluded that RIA is preferable to IRM for measuring TBG in NTI, and that TBG-IRM is a convenient and rapid method to test for thyroid hormone binding inhibitors.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 45004-15 CEB
Formerly Z01 AM 45004

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structure of Polypeptide and Protein Hormones

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H. E. Edelhoch	Senior Scientist	CEB, NIDDK
Others:	R. E. Lippoldt	Health Services Ofcr.-Dir.	CEB, NIDDK
	K. Prasad	Visiting Associate	CEB, NIDDK
	O. Yano	Guest Researcher	CEB, NIDDK

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Protein Structure Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.7	3.6	.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The role of coated vesicles (CVs) in thyroglobulin transport was examined by studies on incorporation of ^{35}S methionine into thyroid slices and examining the thyroglobulin associated with the membranes. The data do not support a primary role for thyroid CVs in either endocytosis or exocytosis of thyroglobulin.

A study of *in vivo* biosynthesis of clathrin and other coated vesicle proteins from rat liver showed that clathrin heavy chains may be recycled during CV formation.

A group of proteins of M_r 100,000-110,000 present in the protein coat of coated vesicles has been shown to be essential for the binding of clathrin to the vesicles. In their absence there was no formation, while adding them back reconstituted the binding nature of clathrin to the vesicles. A protein from the above group has been purified to homogeneity and characterized. It reacts in a stoichiometric ratio with clathrin to form cage-like structures.

An intermediate polymer having a sedimentation coefficient of 27 S has been detected in the assembly of clathrin (8 S) to baskets (150 S).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 45009-19 CEB
Formerly Z01 AM 45009

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies in Thyroid Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. Robbins	Chief, Clinical Endocrinology Branch	CEB, NIDDK
Others:	M. Lakshmanan	Medical Staff Fellow	CEB, NIDDK
	E. Movius	Guest Researcher	CEB, NIDDK
	M. Phyillaier	Biologist	CEB, NIDDK
	J. Norton	Head, Surgical Metab. Section	DCT, NCI
	D. Foti	Visiting Fellow	CEB, NIDDK

COOPERATING UNITS *(if any)*

University of Catania, Italy (Foti)

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Hormone Metabolism and Action Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	1.7	.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Further data have been collected on the usefulness of lithium carbonate as an adjuvant in ^{131}I therapy of thyroid cancer. Although it is effective in some patients in slowing iodine release from the tumor, and thereby increasing its therapeutic effect, its potential role in therapy requires further documentation.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 45014-15 CEB
Formerly AM 45014

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Membranes and Secretion

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. Wolff	Associate Chief	CEB, NIDDK
Others:	D. L. Sackett	Staff Fellow	CEB, NIDDK
	L. Knipling	Technician	CEB, NIDDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Clinical Endocrinology Branch

SECTION

Endocrine Biochemistry Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.4	.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

New data have been obtained on the role of microtubules in modulating cholesterol access to the mitochondrian of adrenal tumor cells in permanent culture. The stimulating effects of colchicine appear not to be mediated via changes in cAMP production as occurs in some cells but is consistent with facilitation of pregnenolone production when microtubules are destabilized and inhibition when they are stabilized by taxol.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 45016-16 CEB
Formerly AM 45016

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Thyroid Hormone Secretion and the Function of Microtubules

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. Wolff	Associate Chief	CEB, NIADDK
Others:	D. L. Sackett	Staff Fellow	CEB, NIADDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Clinical Endocrinology Branch

SECTION

Endocrine Biochemistry Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.3	.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Limited proteolysis of rat brain tubulin yields two types of products, those derived from carboxy terminal cleavage of both subunits and those derived from a single internal cut of each subunit. These changes have major effects on polymerization of tubulin: carboxy terminal cleavage promotes polymerization whereas internal cleavage hinders assembly. The carboxy terminal-cleaved tubulin promotes the assembly of uncleaved tubulin and tissues may use this mechanism to facilitate polymerization under unfavorable conditions.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 45018-11 CEB
Formerly AM 45018

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Adenylate Cyclase and Other Extracellular Products of B. Pertussis

PRINCIPAL INVESTIGATOR *(List other professional personnal below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. Wolff	Associate Chief	CEB, NIDDK
Others:	L. Knipling	Technician	CEB, NIDDK

COOPERATING UNITS *(if any)*
Laboratory of Biochemistry, NCI (D. Newton and Dr. C. Klee)

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Endocrine Biochemistry Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.3	.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Fragments of calmodulin produced by limited trypsin hydrolysis can stimulate the adenylate cyclase of Bordetella pertussis spheroplast membranes in a Ca^{2+}-dependent or Ca^{2+}-independent manner with different potencies. The carboxy terminal fragment (residues 78-148) has high potency, but the cyclase is also stimulated by tryptic fragments 1-77, 1-90 and 107-148. Although very much less potent than calmodulin or fragment 78-148, the activation is not due to contamination by calmodulin but contamination of these fragments by fragment 78-148 is difficult to rule out for lack of an independent biological test. The high affinity Ca^{2+} binding sites probably reside in the carboxy-terminal fragment and the enhancement of the potency produced by Ca^{2+} ions is probably caused by these sites.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 45020-10 CEB
Formerly Z01 AM 45020

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Synthesis of Thyroxine Transport Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. Robbins	Chief	CEB, NIDDK
Others:	M. Phyillaier	Biologist	CEB, NIDDK

COOPERATING UNITS *(if any)*
University of Pisa, Italy (L. Bartalena)

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Hormone Metabolism and Action Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.3	.2	.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

L-asparaginase (ASN), a drug used in the treatment of leukemia, has been reported to decrease specifically the serum thyroxine-binding globulin (TBG) levels. Studies with cultured human hepatoma cells revealed that ASN caused a dose dependent decrease of synthesis of both TBG and albumin, but the former was more sensitive to the drug. An additional effect on the survival of newly synthesized TBG was also observed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 45028-08 CEB
Formerly AM 45028

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Thyroid Hormone-Cell Interactions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. Robbins	Chief	CEB, NIDDK
Others:	M. C. Lakshmanan	Medical Staff Fellow	CEB, NIDDK
	A. Pontecorvi	Visiting Fellow	CEB, NIDDK
	M. Centanni	Guest Researcher	CEB, NIDDK
	M. Phyillaier	Biologist	CEB, NIDDK
	E. Goncalves	Visiting Fellow	CEB, NIDDK

COOPERATING UNITS *(if any)*

University of Rome, Rome, Italy (Pontecorvi, Centanni); University of Porto Allegra, Porto Allegra, Brazil (Goncalves)

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Hormone Metabolism and Action Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.2	3.1	.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The mechanism of transport of thyroid hormones into skeletal muscle cells was studied in two systems, the intact rat soleus muscle and continuously cultured rat myoblasts (L6E9). In intact muscle, uptake of L-T_3 was enhanced by insulin, which appeared to act through the Na^+/H^+ exchanger system. In myoblasts, transport of L-T_3 to cell nuclei was investigated and shown, by comparison with D-T_3, to be regulated by a stereospecific mechanism probably located at the plasma membrane. It was also shown, by the use of specific inhibitors, that the transfer of T_3 from plasma to the cell nucleus is temperature dependent, requires energy and an intact cytoskeletal architecture, and is probably mediated through an endocytotic pathway. These results indicate further that thyroid hormone uptake into cells may be under metabolic control.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 45033-03 CEB
Formerly AM 45033

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mapping of Triiodothyronine Responsive Genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	V. M. Nikodem	Expert	CEB, NIDDK
Others:	H. Morioka	Guest Researcher	CEB, NIDDK
	S. Usala	Medical Staff Fellow	CEB, NIDDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Hormone Metabolism and Action Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	1.5	.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

1. The genomic clone containing the putative promoter region of rat malic enzyme gene has been obtained and sequenced. None of the expected consensus sequences (TATA and CAAT box) were found. Instead, this region is G/C rich and contains in direct repeat six CCGCCC hexanucleotides upstream of the transcription site. Several deletion mutations at this region were analyzed for a promoter activity by calcium phosphate mediated transfection into Chinese hamster ovary cells. Preliminary results show that -165 base pairs can direct the transient expression of the structural part of the CAT gene, while -40 and -85 base pairs are insufficient.

2. The chromatin structure of the malic enzyme gene has been analyzed in different thyroidal states. No differences in methylation patterns were detected after thyroid hormone treatment. It appears that a number of hypersensitive sites at the 5' and 3' end of this gene do not depend on thyroid hormone, but some of these sites are substantially stronger in hyperthyroid animals. However, a great majority of the malic enzyme gene does not demonstrate hypersensitivity sites. This suggests heterogeneity of malic enzyme gene activity in hepatocytes, resulting from a different functional capacity of individual hepatocytes to synthesize malic enzyme. Presently, we are investigating this possibility.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 45034-03 CEB
Formerly AM 45034

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Specific Rat Liver mRNAs by Thyroid Hormone

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, laboretory, and institute affiliation)*

PI:	V. M. Nikodem	Expert	CEB, NIDDK
Others:	M. A. Magnuson	Medical Staff Fellow	CEB, NIDDK
	B. Dozin	Visiting Fellow	CEB, NIDDK
	M. Tecce	Visiting Fellow	CEB, NIDDK
	J. Rall	Dep. Dir., Intramural Res.	OD, NIH

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Clinical Endocrinology Branch

SECTION

Hormone Metabolism and Action Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	1.2	.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use stenderd unreduced type. Do not exceed the space provided.)*

1. We have studied and sequenced a thyroid hormone-regulated rat liver mRNA (4-12B) and showed that it is related to the superfamily of serine protease inhibitors, with the highest similarity to mouse contrapsin. The optimized alignment with the related sequences indicates that lysine-serine residues are located at the reactive site or adjacent to it. This suggests that 4-12B mRNA could code for the protein contributing to the serum trypsin inhibiting activity in rat. These protease inhibitors control proteolytic degradation responsible for immune reactions, coagulation and inflammation. This finding, that a protease inhibitor is transcriptionally regulated by T_3, could represent a previously unrecognized mechanism by which thyroid hormone might affect any of several important physiological processes.

2. Regulation of malic enzyme mRNA by a high carbohydrate diet is liver specific. Furthermore, the amplitude of the response depends on the thyroidal state of the animal, being lower by a factor of ~ 4 in hypothyroidism and higher in hyperthyroidism. Mathematical modeling shows that this increase in cytoplasmic mRNA is compatible with retarded degradation of cytoplasmic mRNA while thyroid hormone regulates the synthesis of this mRNA predominantly at the nuclear level.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 45035-03 CEB
Formerly AM 45035

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Photoaffinity Labeling of Thyroid Hormone-Specific Binding Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	V. M. Nikodem	Expert	CEB, NIDDK
Others:	H. J. Cahnmann	Scientist Emeritus	CEB, NIDDK
	D. G. Sheer	Staff Fellow	CEB, NIDDK

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Hormone Metabolism and Action Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.9	1.8	.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The putative thyroid hormone receptor(s) from rat liver nuclei could be covalently photolabeled with underivatized hormone. When 0.4 M NaCl extract from purified nuclei is labeled, two major binding proteins (57 and 46 kDa) are resolved on SDS gel. However, radioactivity ratios vary considerably, ranging from predominance to absence of 57 kDa protein. Limited proteolysis of these two major binding proteins yielded virtually identical patterns. This is indicative of a high sensitivity of 57 kDa protein to breakdown in the course of sample preparation. To eliminate such a possible brakdown, we introduced the following modifications to our previously used procedure: 1) use of glycerol in all buffers; 2) increase of Mg^{2+} concentration; 3) reduction of irradiation time from 30 min to 1 min; 4) introduction of an acetone precipitation of labeled nuclear proteins. These modifications resulted in incorporation of radioactivity in the ~ 57 kDa protein (resolved as a doublet) while incorporation in the 46 kDa protein became minimal. Thus, it appears that a high concentration of glycerol, magnesium and a shorter time of irradiation stabilize the doublet (60, 57 kDa). The presence of this doublet was also detected in nuclear extracts from heart and brain while testis and spleen showed only a trace of it. This is in agreement with the absence of high affinity binding sites for thyroid hormone in testis and spleen as determined by Scatchard analysis. Hence, the endogenous thyroid hormone receptor has a molecular weight of about 60 kDa. Furthermore, preliminary results showed that the high molecular weight thyroid hormone binding protein binds to calf thymus DNA and is eluted by a relatively high concentration of salt.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 AM 45036-02 CEB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regulation of Malic Enzyme Gene Expression in Cultured Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	V. M. Nikodem	Expert	CEB, NIDDK
Others:	G. S. DeCherney	Guest Researcher	CEB, NIDDK

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Hormone Metabolism and Action Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Terminated September 30, 1985

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 45037-01 CEB

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Hormones and Cell Differentiation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Pontecorvi	Visiting Fellow	CEB, NIDDK
Others:	V. M. Nikodem	Expert	CEB, NIDDK
	M. Phyillaier	Biologist	CEB, NIDDK
	J. Robbins	Chief	CEB, NIDDK
	J. Tata	Fogarty Scholar	FIC, NIH

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Clinical Endocrinology Branch

SECTION

Hormone Metabolism and Action Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	1.0	.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The differentiation of rat myoblast cultured cells, L6A1R5, is stimulated by insulin and is accompanied by an increase in creatine kinase (CK) activity. Creatine kinase mRNA was measured by hybridization with creatine kinase cDNA. A dose of insulin that caused a 203 fold increase in CK activity produced only a 17.5 fold increase in CK mRNA. This suggests that insulin has two effects, an increase in mRNA levels and an additional effect on the rate of protein synthesis or processing.

GPO 914-918

ANNUAL REPORT OF THE DIABETES BRANCH
NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

Recognition of Previous Achievements

The Diabetes Branch continues to pursue a broad based program which encompasses clinical research, studies on the mechanism of insulin action, with special emphasis on the nature and function of the insulin receptor, studies on the evolution of hormones and their function as messenger molecules, studies on morphological interaction of hormones with cells, and detailed studies of the biosynthesis of the insulin receptor.

Grants were made to members of the Diabetes Branch from both the Juvenile Diabetes Foundation and the American Diabetes Association. An additional fellowship was received from the Pharmacology Research Associate Program of the National Institute of General Medical Sciences.

Dr. Emmanuel Van Obberghen (Diabetes Branch alumnus) was awarded the Minkowski Award of the European Diabetes Association. Dr. Phillip Gorden was awarded a doctorate Honoris Causa from the University of Geneva and the Distinguished Service Medal of the United States Public Health Service.

INSULIN RECEPTORS AND RELATED HORMONES

Phosphorylation of the Insulin Receptor

It is now clear that the alpha-subunit of the insulin receptor is the major binding subunit of the receptor and that the beta-subunit is a protein kinase, capable of autophosphorylating itself and a number of exogenous substrates. Many details of this phosphorylation reaction in both blood cells and other cells have been under continued study. Recent studies have been directed at the nature of phosphorylation in brain receptors versus peripheral receptors such as liver. It is clear that each tissue receptor has both a binding and protein kinase region and in that sense, all insulin receptors are similar; they are somewhat different, however, in their migration on gels. In studies carried out on guinea pigs, chickens, rats, lizards, frogs, and alligators, it is clear that these differences in structure are maintained both ontogenetically and phylogenetically. Further, similar studies have been carried out in

neuroblastoma cell lines from embryonic, neonatal and adult tissues of the rat as well as in primary neuronal and glial cell cultures from rat. These studies expand and extend the initial studies from whole animal brain.

It is further known that the tyrosine kinase activity of the insulin receptor is very similar to the tyrosine kinase activity for several other growth factor receptors and oncogene proteins. When an antibody raised against $pp60^{src}$ was reacted with the insulin receptor, it was shown that this antibody would immunoprecipitate the insulin receptor, thus, demonstrating the similarity in the protein kinase region between the src phosphoprotein and the insulin receptor phosphoprotein.

It is clear that the beta-subunit of the insulin receptor is a tyrosine-specific protein kinase capable of autophosphorylating itself or other artificial substrates. In additional studies we have shown that an endogenous substrate present in the 120 kDa glycoprotein membrane fraction of hepatocytes is also phosphorylated in a tyrosine-specific manner. In addition to serving as a substrate for the insulin receptor, pp120 can be phosphorylated by the hepatic epidermal growth factor receptor. Most importantly it has been recently shown that insulin stimulates phosphorylation of tyrosine residues of pp120 in intact hepatoma cells.

In an attempt to study the tyrosine kinase activity of the insulin receptor in a more quantitative fashion, we have developed a new technique using red blood cells. The technique primarily employs the use of red blood cell ghosts and ricin columns to partially purify the receptor. Under these conditions, the receptor can be incubated with artificial substrates as previously shown with monocytes. This creates a more sensitive assay and we have been able to show an approximately 40% decrease in the coupling of alpha and beta subunit function in Type II diabetics as compared to normal controls.

We have previously shown that tumor-promoting phorbol esters which are known to be serine kinases in other cell types can stimulate specific tyrosine kinase activity in peripheral blood monocytes and in U-937 monocyte cell lines. Since these tumor-promoters decrease the affinity of insulin for its receptor, we have tested diacylglycerols which are known to simulate the effects of phorbol esters. We find that diacylglycerol as the phorbol ester inhibits insulin binding to IM-9 and U-937 cells. In addition diacylglycerols and phorbol esters stimulate a complex form of

phosphorylation depending on whether one is studying cell-free systems or intact cell systems. In an attempt to sort out this process we are continuing our studies.

Cellular Hormone-Like Peptides and Embryo Receptors

Insulin-related peptides have been isolated from the unicellular eukaryotic organism Tetrahymena, bacteria including E. coli and plant extracts. Additional studies are in progress to characterize these peptides. Extensive characterization of insulin-related material in higher plants has been performed. At this point, the insulin-related materials appear to be typical of mammalian type insulin by HPLC characterization, immunoactivity, and now biological activity studies. Two specific differences, however, have been demonstrated for the insulin-like material, i.e. it can be immunologically distinguished from mammalian or avian insulins and its elution pattern on HPLC is different from other known insulins. Also studies are now in progress to identify the genes for these peptides in unicellular organisms. Initial studies have demonstrated hybridization with an insulin probe as well as a somatostatin probe. Studies are in progress in an attempt to clone the DNA responsible for this hybridization reaction.

Receptor studies have been continued using "chicken embryo" as the model; at the earliest stages of development growth factor, i.e. IGF-I, receptors appear to predominate. Insulin receptors at a later stage of development increase linearly as a function of embryo age. Additional studies have attempted to define the role of insulin in early embryonic development. The initial findings demonstrate that insulin antibodies which inactivate insulin are associated with a greater degree of growth retardation.

Insulin receptor structural studies and regional localization have been extended in rat brain. The binding of labeled insulin to thin sections of frozen fresh rat brain was visualized by autoradiography. The labeled insulin binding sites in rat brain formed a distinct pattern with high levels of binding in all olfactory areas and in closely related limbic regions. Binding was also prominent in the neocortex and the assessory motor areas of the basal ganglia and the cerebellum. Enrichment of insulin receptors in olfactory and limbic areas is characteristic of other known neuropeptides which suggest a neuromodulary function for insulin in the brain.

Morphologic Studies of Ligand Binding to Cells

These investigations are a continuing collaboration with the University of Geneva. The monocyte had been used as a major indicator of insulin receptor function in the whole body. It is now clear that the monocyte insulin receptor is regulated in a very similar manner to the rodent hepatocyte receptor. Thus, monocytes internalize insulin in a qualitatively identical manner to hepatocytes. This suggests that the monocyte is being controlled by the same external factors as is a major target tissue. Other studies have attempted to assess the relationship between binding of a hormone to the cell surface and its dissociation rate. We find that there is a direct association between slowing dissociation of ^{125}I-insulin and a redistribution of the hormone from the villous surface of the cell to the non-villous and coated surface of the cell.

Further studies have attempted to study the relationship between endocytosis and recycling. The U-937 cell internalizes insulin readily and insulin down-regulates its receptor as would be expected. Monensin, a drug which inhibits recycling, enhances this effect of insulin down-regulation, thus suggesting that recycling is an important adjunct to internalization. By contrast, the IM-9 lymphocyte which internalizes insulin poorly, shows essentially no additional effect when monensin is added. Thus it appears that cells that internalize receptors to a high degree recycle receptors to a significant degree.

Since receptor-mediated endocytosis is an important mechanism by which cell surface receptors are regulated, we investigated the question of whether there is regulation in hypoinsulinemic states. We find in hepatocytes from streptozotocin-treated rats and in monocytes from Type I diabetics patients that there is a marked inhibition of internalization of ^{125}I-insulin suggesting that there is a regulated mechanism designed to preserve the ligand on the cell surface in order to amplify a muted signal.

Intracellular calcium appears to play no essential role in the internalization of receptor-bound ligands. Protein kinase C, as stimulated by phorbol ester, on the other hand, appears to have a major role in stimulated internalization of two very different ligands such as insulin and transferrin, though there are some differences in the properties of these two ligands.

Other studies have attempted to more clearly define the endosomal compartment of the rodent hepatocyte. By using cholchicine, the transfer of ligands in the cell surface to lysosomes can be inhibited suggesting that the role of this intermediate vesicular compartment is more clearly delineated.

Insulin Receptors in Syndromes of Extreme Insulin Resistance

Insulin receptor biosynthetic studies have been carried out using tritiated sugars and amino acids. One study revealed a defect that showed a decrease in the precursor for the receptor and the mature receptor components, however, other studies indicate that additional defects are emerging. The use of a cloned cDNA probe for the insulin receptor in an attempt to study the earliest events in receptor biosynthesis is being incorporated into this project.

Clinical studies in patients with the syndromes of extreme insulin resistance have revealed examples of genetic compounds i.e., one gene is presumably inherited from the mother and one gene from the father. The mother and the father may, themselves, appear phenotypically normal but a compound situation results in the mutant phenotype of the offspring.

Patients with insulin resistance have continued to be studied with regard to their insulin receptor. Both peripheral blood monocytes, erythrocytes, as well as fibroblasts and transformed lymphocytes have been utilized in the study. In previous studies we have demonstrated a marked discordance between alpha-subunit (binding function) and beta-subunit (protein kinase function) in one patient with the Type A syndrome of insulin resistance. These studies have now been extended to show that this dicotomy also exists in erythrocytes and fibroblasts. Interestingly, however, the defect is repaired in EB virus-transformed lymphocytes. The mechanism for this difference is as yet unclear. In other patients with the typical Type A syndrome of insulin resistance and low alpha-subunit function, tyrosine kinase activity is generally concomittantly low.

Autoantibodies to the Insulin Receptor

Further studies are in progress to analyze the characteristics of spontaneously occurring anti-insulin receptor antibodies from different patients who manifest different clinical syndromes of either hypoglycemia or insulin resistance. Other studies have used antibodies to specific

regions of protein such as the src protein to show overlapping sequences between the pp60^{src} protein and the kinase area of the insulin receptor.

Growth Hormone and Acromegaly

Using standard cross-linking techniques, the growth hormone receptor of the IM-9 lymphocyte has been identified as an approximately 140 kDa protein, under reducing conditions. Under non-reducing conditions the receptor also exists as a 270 kDa and a minor 230 kDa band on polyacrylamide gel electrophoresis. Studies to-date have shown that unlike the insulin receptor, growth hormone receptor is not autophosphorylated and that it probably exists as a homodimer between respective receptor molecules. Additional studies have identified the growth hormone receptor as a glycoprotein. These results demonstrate that the receptor is effected by neuraminidase treatment, is effected by enzymes that cleave N-linked sugars from the nacent protein. There is a distinction, however, in the amount of high mannose form of carbohydrate between the growth hormone and the insulin receptor. Thus the growth hormone receptor seems to have more complex carbohydrates in the mature receptor than does the insulin receptor. More recent studies have been aimed at attempting to immunoprecipitate the growth hormone receptor for biosynthetic studies.

Therapeutic studies in acromegaly have been continued in three ways: a) surgical therapy, b) conventional supervoltage irradiation and, c) pharmacologic treatment. The most recent pharmacologic treatment applied is a new somatostatin analogue (SMS 201-995). We have used this analogue in treating acromegalic patients and in individuals with TSH-secreting tumors. The drug appears to be effective in a limited number of patients whose growth hormone is sufficiently low to allow for sustained growth hormone suppression. Further studies with this agent are in progress.

Biosynthetic Labeling of the Insulin Receptor

Using both labeled sugars and amino acids, the life cycle of the insulin receptor has been studied. First, it is clear that the receptor is initially synthesized as a 190 kDa protein. This has now been confirmed by structural studies derived from cDNA cloning techniques. In addition, the structural studies confirmed that the alpha-subunit is externally exposed on the plasma membrane whereas the beta-subunit is a

transmembrane protein exposed both on the external and cytoplasmic face of the plasma membrane. Thus the biosynthetic studies and receptor orientation studies derived from cDNA cloning results have now been confirmed. This model assumes that the amino acid backbone of the receptor is translated in the endoplasmic reticulum of the cell where the high mannose core of the glycoprotein is added co-translationally to the nacent basal peptide which results in the 190 kDa pro-receptor. This proreceptor is then further processed in the Golgi region of the cell by cleavage and complex glycosylation.

In an attempt to define the role of the glucose moieties of the high mannose carbohydrate, the inhibitor 1-deoxynojirimycin was employed along with castanospermine to inhibit the glucosidase activity. We find that this markedly retards the insertion of the mature receptor into the plasma membrane. Further, HPLC studies confirm that the glucose moieties remain intact on the high mannose carbohydrate. Other HPLC studies have characterized the nature of the endo-H cleavable carbohydrate from the insulin pro-receptor and confirm that this is a classic high mannose form.

Our most recent studies have demonstrated a new and heretofore unknown post-translational modification of the insulin receptor, i.e. fatty acylation. Thus, both myristic and palmitic acid are added to both alpha and beta components of the insulin receptor during post-translational processing.

One important question related to insulin action is how can insulin receptor concentrations be increased on the cell surface? It is clear that an increase in the cell surface receptor concentration would enhance insulin action. Recent studies have demonstrated that corticosteroids increase the cell surface receptor concentration. Previously we have shown that this was not due to stabilization of the receptor but that pro-receptor synthesis is enhanced by the corticosteroids. This demonstrates directly that corticosteroids are capable of stimulating the synthesis of the insulin receptor. This is the first pharmacologic agent known to serve this function.

Cultured Cell Models for Hormone Receptors

In an attempt to understand the details of defects in cell lines derived from patients, we have studied model systems that have defined abnormalities. The principal cell that has been studied is the cultured Chinese hamster ovary cells. In addition to the wild-type cell, two

mutant cell lines have been studied. One mutant cell line has a defect in the biosynthesis of dolichol and transfers a shortened high mannose form of the carbohydrate which is subsequently processed. This mutant cell binds insulin with a higher affinity than the wild-type cell. The second mutant cell that has been studied has a defect in complex glycosylation. This mutant cell binds insulin with a much lower affinity. Studies using lectin probes have verified the altered carbohydrate composition of the Lec 1 cells and have generally shown that the B4-2-1, i.e. the high affinity binding cell, have a structure similar to the wild-type cells. Other studies have demonstrated that the growth media, i.e. the metabolic milieu of the cell, can dictate the binding affinity of the receptor. Thus the wild-type cell can be made to express the phenotype of the high affinity binding B4-2-1 cell when grown in a glucose-deficient media. Thus, it is clear that both metabolic manipulation and genetic-enzymatic manipulation can both operate to control the affinity of the insulin receptor.

In contrast to the insulin receptor, the IGF-I receptor is essentially normal in the mutant cell lines. It has abnormal mobility on gels as does the insulin receptor, but its binding parameters are entirely within normal limits. Similarly, IGF-I binding tends to be normal in fibroblasts derived from patients with syndromes of insulin resistance. This is, again, in contrast to the insulin binding which is markedly abnormal. Further, a specific IGF-I antibody is capable of partially inhibiting ^{125}I-insulin to these cells, suggesting an abnormal structural characteristic of their insulin receptors.

Since liver cells have been extensively studied with respect to the insulin receptor and insulin action, we characterized a human liver cell line, the Hep G-2 cell. This cell binds, internalizes, and processes insulin in an identical fashion to what has been previously shown for rodent cell lines. It has the advantage of degrading insulin in a more normal fashion to freshly isolated hepatocytes. Further a number of biological effects of insulin have been demonstrated in this cell line.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 47001-05 DB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Phosphorylation of the Insulin Receptor

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	D. LeRoith	Section Chief	DB, NIDDK
Others:	J. Simon	Guest Researcher	DB, NIDDK
	W. Lowe	Medical Staff Fellow	DB, NIDDK
	J. Shemer	Guest Researcher	DB, NIDDK
	C. Hart	Guest Researcher	DB, NIDDK
	J. Roth	Director, DIR	NIDDK
	M. Raizada	Associate Professor	Gainesville, FL
	R. Pruss	Senior Staff Fellow	NIMH

COOPERATING UNITS *(If any)*
Department of Physiology, Gainesville, Florida

LAB/BRANCH
Diabetes Branch

SECTION
Receptor and Hormone Action

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 5.0	PROFESSIONAL: 5.0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The insulin receptor is a heterotetrameric membrane bound glycoprotein composed of two predominantly extracellular alpha subunits that bind insulin and two beta subunits that span the membrane and are capable of demonstrating autophosphorylation as well as containing tyrosine kinase activity in their cytoplasmic domain.

Brain insulin receptors are smaller than non-neural insulin receptors, as seen on sodium dodecyl sulphate polyacrylamide gel electrophoresis. This difference in molecular weight is mainly due to differences in glycosylation as demonstrated by insensitivity of brain insulin receptors to neuraminidase digestion. Phylogenetic and ontogenetic studies demonstrated that the difference in brain insulin receptors is evolutionarily and developmentally well conserved. Despite these structural differences, brain insulin receptors, like their non-neural counterparts, are capable of undergoing insulin-induced auto-phosphorylation of the beta subunit as well as insulin-stimulated phos-phorylation of tyrosine specific artificial substrates. Thus insulin receptors on nervous tissue are capable of responding normally to insulin.

Formerly Project Number Z01 AM 47001-04 DB

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 47005-14 DB

PERIOD COVERED: October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Studies of Insulin Receptors in Circulating Cells in Man

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	R. J. Comi	Medical Staff Fellow	DB, NIDDK
Others:	S. I. Taylor	Medical Officer	DB, NIDDK
	J. L. Young	Biol. Lab. Tech.	DB, NIDDK
	P. Gorden	Chief	DB, NIDDK

COOPERATING UNITS (if any): None

LAB/BRANCH: Diabetes Branch

SECTION: Clinical and Cellular Biology Section; Biochemistry and Molecular Pathophysiology Section

INSTITUTE AND LOCATION: NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
- [X] (b) Human tissues
- [ ] (c) Neither
- [X] (a1) Minors
- [ ] (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The present work has concentrated primarily on studying the tyrosine kinase activity of the insulin receptor. In previous studies of a mutant cell line from an insulin resistant patient, a defect in tyrosine kinase activity in the monocytes was demonstrated. A similar defect can be shown in erythrocytes and fibroblasts and repair of this defect can be found in EBV-transformed lymphocytes from the patient. In contrast, most patients with Type A syndrome with insulin resistance and low binding have concomitantly low tyrosine kinase activity. A new method has been developed to study red blood cell tyrosine kinase activity of the insulin receptor. A defect in Type II diabetes can be shown. Studies are underway to determine the effects of fasting, diet and treatment on this defect. Diacylglycerol, a phorbol ester, also inhibits insulin binding to cultured cells and blood monocytes. Further these tumor-promoting agents cause a complex-type of phosphorylation of the insulin receptor.

FORMERLY Z01 AM 47005-13 DB

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DK-47007-11 DB

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Antibodies to Receptors: Detection in Disease States and Use As Probes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. : S. I. Taylor Chief, Bio. & Mol. Path. Section DB/NIDDK

Others:	N. Perrotti	V.A.	DB/NIDDK	R. DePirro	Prof.	Italy
	J. Roth	Director	DB/NIDDK	N. Richert	V.A.	NCI/NIH
	B. Samuels	Chemist	DB/NIDDK			
	S. Fuchs	V.S.	DB/NIDDK			
	G. Grunberger	S.C.A.	DB/NIDDK			
	V. Moncada	Biologist	DB/NIDDK			

COOPERATING UNITS *(if any)*

University of Rome, Rome, Italy (Dr. R. De Pirro) - Foreign
Division of Cancer Biology and Diagnosis (Dr. N. Richert, Visiting Associate)
NCI, NIH

LAB/BRANCH

Diabetes Branch

SECTION

Biochemistry and Molecular Pathophysiology Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Antibodies directed against the insulin receptor have been invaluable reagents in the investigation of receptor structure and function. Initially, these anti-receptor antibodies were identified as spontaneously appearing autoantibodies in patients with autoimmune disease associated with either extreme insulin resistance or hypoglycemia. The utility of these anti-receptor antibodies is limited to some extent because of several factors. Most importantly, it is not known which epitopes on the receptor are recognized by these antibodies. Consequently, we have recently attempted to develop antibodies which would recognize specific sites on the insulin receptor. Toward that end, we have immunized rabbits with peptides corresponding to partial amino acid sequences of the receptor. In addition, we have employed antibodies directed against pp60^{src} to demonstrate structural homology between the insulin receptor and tyrosine kinases encoded by viral oncogenes.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 47014-17 DB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Acromegaly and Growth Hormone

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)*

P.I.:	P. Gorden	Chief	DB, NIDDK
Others:	K. Asakawa	Visiting Associate	DB, NIDDK
	C. M. Hendricks	Biol. Lab. Tech.	DB, NIDDK
	G. Grunberger	Clinical Associate	DB, NIDDK
	A. McElduff	Visiting Associate	DB, NIDDK
	J. A. Hedo	Visiting Associate	DB, NIDDK

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Diabetes Branch

SECTION
Clinical and Cellular Biology Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.2	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Acromegalic patients have continued to be followed with respect to pituitary irradiation. Further, we are evaluating the effects of transsphenoidal hypophysectomy in these patients and comparing them to the pituitary-irradiated patients.

Recently, human growth hormone has been cross-linked to its specific cellular receptor on IM-9 cultured lymphocytes. Under reducing conditions the predominant band is an approximate 140K protein. Under non-reducing conditions, however, a molecular weight component of ~ 270K is observed. Further attempts were made to see if the growth hormone receptor was a protein kinase or a substrate for this kinase activity; it is not. Thus, the growth hormone receptor is not analogous to the insulin receptor.

The heterogeneity of circulating growth hormone in plasma has been studied. Pituitary growth hormone was injected in normal volunteers and the individual growth hormone components isolated by gel filtration. It was found that the half-time of the "little" (22,000 Dalton) growth hormone component was faster than the "big" and the "pre-big" growth hormone components, respectively. This is compatible with a receptor-mediated type of removal of these components; previous studies have shown that the high molecular weight forms have lower radioreceptor activity than the 22,000 Dalton growth hormone preparation.

FORMERLY Z01 AM 47014-16 DB

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 47018 09 DB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cellular Hormone-like Peptides

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	J. Roth	Scien. Dir.	DB, NIDDK
Others:	D. LeRoith	Section Chief	DB, NIDDK
	M.A. Lesniak	Chemist	DB, NIDDK
	E. Collier	Medical Staff Fellow	DB, NIDDK
	C. Roberts	Expert	DB, NIDDK
	G.L. Wilson	Biologist	DB, NIDDK
	J. Shiloach	Visiting Scientist	A, LCDB
	C. Cleland		Smithsonian

COOPERATING UNITS *(if any)*

Laboratory of Cellular and Developmental Biology, NIDDK
Smithsonian Institute (Rockville)

LAB/BRANCH
Diabetes Branch

SECTION
Receptors and Hormone Action Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.0	4.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Materials closely resembling vertebrate-type peptide hormones (insulin, somatostatin, adrenal-corticotrophin hormone, relaxin, calcitonin, arginine-vasotocin) have been identified in unicellular organisms. These studies were extended to include a search for similar peptides in plants. Insulin and somatostatin-related materials were found in extracts of spinach, lemna and rye. Purification of the peptides was achieved using gel chromatography and high performance liquid chromatography and activity demonstrated by the specific hormone radioimmunoassays and bioassays.

Using recombinant DNA technology, the genes encoding for insulin and somatostatin were searched for in genomic extracts from unicellular eukaryotes and prokaryotes as well as Drosophila. Specific DNA sequences were found which hybridized to cDNA probes for rat somatostatin and rat insulin and are currently being analyzed for sequence composition. Clones with lambda gt11 expression were probed with anti-insulin and anti-somatostatin antibodies. Positive clones are being processed for nucleotide sequences. These results suggest that hormone-like peptides are evolutionarily very old.

Formerly Project Number Z01 AM 47018 08 DB

PHS 6040 (Rev 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 47019-09 DB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Morphologic Studies of Ligand Binding to Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	P. Gorden	Chief	DB, NIDDK
Others:	G. Grunberger	Clinical Associate	DB, NIDDK
	J.-L. Carpentier	Research Associate	Geneva, Switzerland
	A. Robert	Research Associate	Geneva, Switzerland
	L. Orci	Professor of Medicine	Geneva, Switzerland

COOPERATING UNITS *(if any)*
Institute of Histology and Embryology, University of Geneva School of Medicine, Geneva, Switzerland

LAB/BRANCH
Diabetes Branch

SECTION
Clinical and Cellular Biology Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The U-937 monocyte-like cultured cell, a model for circulating monocytes, binds and internalizes insulin in an analogous fashion to target cells such as liver cells. The U-937 monocyte internalizes insulin at a high rate and the addition of monensin to insulin markedly augments down regulation of receptors. By contrast, the IM-9 lymphocyte which internalizes insulin poorly, shows essentially no additional effect when monensin is added. The drug colchicine which inhibits endosomal transfer to lysosomes in hepatocytes has been studied. There is no localization to an additional compartment such as the Golgi with respect to processing of an internalized ligand. The internal route appears to go from the endosome of one or more types to the lysosome of one or more types; the recycling process would appear to occur from either of the two membrane-bounded structures.

FORMERLY Z01 AM 47019-08 DB

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 47021-08 DB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cultured Cell Model for Hormone Receptors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	J. M. Podskalny	Biologist	DB, NIDDK
Others:	D. G. Rouiller	Visiting Associate	DB, NIDDK
	G. Grunberger	Clinical Associate	DB, NIDDK
	P. Gorden	Chief	DB, NIDDK

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Diabetes Branch

SECTION
Clinical and Cellular Biology Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	3.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The insulin-like growth factor-I (IGF-I) receptor of Chinese hamster ovary (CHO) cells has been characterized in wild type and two mutant CHO lines. The oligosaccharide portion of the IGF-I receptor and/or other non-receptor glycoproteins does not appear to affect IGF-I receptor affinity. This is in sharp contrast to the situation with insulin receptors which appear to be significantly affected by glycosylation changes. Glucose starvation, which forces the wild type cells to become phenotypically identical to one of the mutant cell lines, has no effect on IGF-I binding while it significantly increased insulin binding due to an increase in affinity. A monoclonal antibody to the human IGF-I receptor, alpha-IR3, reacts with the hamster IGF-I receptor equally well in all CHO lines. By using CHO cells, both wild type and mutants, and various lectins, we have determined indirectly that the carbohydrate units of the insulin receptor are heterogenous. Some insulin receptor carbohydrates may play a role at cell surface while others may express more intrinsic properties. In studies with fibroblasts cultured from patients with severe insulin resistance, significantly elevated levels of IGF-I binding was found in 3 of 8 patients with lipoatrophic diabetes. Fibroblasts from an infant with leprechaunism and her phenotypically normal mother both had normal levels of IGF-I binding despite their abnormal insulin binding. Monoclonal antibody alpha-IR3 was able to partially inhibit ^{125}I-insulin binding to both cell lines and the presence of alpha-IR3 in insulin competition curves shifted the dose response curve to the right.

FORMERLY Z01 AM 47021-07 DB

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DK-47022-07 DB

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Insulin Receptors in Syndromes of Extreme Insulin Resistance

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. : S.I. Taylor Chief, Bio. & Mol. Path. Section DB/NIDDK

Others:	K. Ojamaa	V. F.	DB/NIDDK	J. Young	B.	DB/NIDDK
	B. Samuels	Chemist	DB/NIDDK	G. Grunberger	S.C.A.	DB/NIDDK
	V. Moncada	Biochemist	DB/NIDDK	R. Comi	M.S.F.	DB/NIDDK
	A. Cama	V.F.	DB/NIDDK	J.A. Hedo	V.A.	DB/NIDDK
	A. Greenberg	M.S.F.	DB/NIDDK	P. Gorden	Chief	DB/NIDDK
	D. Accili	V.F.	DB/NIDDK	M. Serrano-Rios	Prof.	SPAIN
				A. Ullrich	Prof.	CALIFORNIA

COOPERATING UNITS *(if any)*

Madrid, Spain, (Dr. M. Serrano-Rios) - Foreign
Genetech, Inc., South San Francisco, CA. (Dr. A. Ullrich) - U.S.

LAB/BRANCH

Diabetes Branch

SECTION

Biochemistry and Molecular Pathophysiology Section

INSTITUTE AND LOCATION

NIDDK, NIH Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.0	5.5	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Multiple different biochemical defects have been identified in patients with genetic forms of extreme insulin resistance. These defects have included: (1) a decreased number of insulin receptors, (2) qualitatively abnormal insulin receptors which are impaired in their ability to couple insulin binding to insulin action, and (3) post-receptor defects in insulin action.

We have applied the methods of cell biology to further define the nature of the receptor defects. Using cultured cells derived from insulin resistant patients with a decrease in receptor number, we have identified several different types of defects in the pathway of receptor biosynthesis. In a patient with qualitative abnormalities in insulin binding, we have obtained evidence suggesting that the patient is a genetic compound, having inherited two distinct mutant alleles which impair receptor function. In addition, with monocytes from a patient whose receptor-associated tyrosine kinase activity is defective, evidence has been obtained which suggests an abnormality in the molecular weight of the alpha-subunit of the insulin receptor.

Most recently, in collaboration with Dr. Axel Ullrich, we have begun to apply recombinant DNA technology to determine whether there are mutations in the insulin receptor genes of these patients.

In a related project, we have embarked upon studies to identify the cause of insulin resistance in an animal model of obesity and diabetes (the ob/ob mouse). Preliminary studies suggest that adenylate cyclase fat cells from ob/ob mice have abnormally increased sensitivity to adenosine.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 47024-07 DB

PERIOD COVERED: October 1, 1985 to September 30, 1986

TITLE OF PROJECT (60 characters or less. Title must fit on one line between the borders.)
Biosynthetic Labeling of the Insulin Receptor

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	J. A. Hedo	Visiting Associate	DB, NIDDK
Others:	I. A. Simpson	Visiting Scientist	MCNEB, NIDDK
	R. F. Arakaki	Medical Staff Fellow	DB, NIDDK
	D. G. Rouiller	Visiting Associate	DB, NIDDK
	A. McElduff	Visiting Associate	DB, NIDDK

COOPERATING UNITS (if any)
Experimental Diabetes, Metabolism and Nutrition Section, Molecular, Cellular and Nutritional Endocrinology Branch

LAB/BRANCH
Diabetes Branch

SECTION
Clinical and Cellular Biology Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have studied the biosynthesis of the insulin receptor in human IM-9 lymphocytes and isolated rat adipocytes. The alpha (M_r=135,000) and beta (M_r=95,000) subunits of the receptor are synthesized in the endoplasmic reticulum as a M_r=190,000 single polypeptide, high mannose glycoprotein. This proreceptor is then transported to the Golgi apparatus where it undergoes proteolytic cleavage and carbohydrate processing. Direct analysis by high performance liquid chromatography of the carbohydrate chains of the insulin proreceptor demonstrate that the largest oligosaccharide present is $Glc_1Man_9GlcNAc_2$. This structure represents only a small fraction (3%) of the total. The predominant proreceptor oligosaccharides are $Man_9GlcNAc_2$ (25%) and $Man_8GlcNAc_2$ (48%). Assuming that a $Glc_3Man_9GlcNAc_2$ species is transferred co-translationally, carbohydrate processing of the proreceptor appears to be very rapid and limited to the removal of three glucoses and one mannose residue. We have also investigated the role of this early carbohydrate processing by treating cells with two different inhibitors of glucosidases: castanospermine and 1-deoxynojirimycin. In the presence of these agents an abnormal precursor (M_r=205,000) is synthesized. The processing of this proreceptor to mature subunits is markedly delayed and there is a reduction of cell surface insulin receptors. Thus glucose removal is probably an important signal for further processing and transport of the receptor. In addition the regulation of the proreceptor synthesis has been studied. Hydrocortisone treatment of IM-9 lymphocytes induces a 2-3 fold increase in the proreceptor concentration which leads to a similar increase in cell surface receptors.

FORMERLY Z01 AM 47024-06 DB

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-DK-47025-03 DB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Tissue Receptors for Insulin and Insulin-like Growth Factors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	M.A. Lesniak	Chemist	DB/NIDDK
Others:	L. Bassas	Guest Worker	DB/NIDDK
	J. Roth	Scientific Director	DB/NIDDK
	J. Hill	Visiting Associate	DB/NIDDK
	C. Pert	Section Chief	CNB/NIMH
	F. de Pablo	Visiting Scientist	LCDB/NIDDK
	M. Girbau	Chemist	Univ. Sao Paolo

COOPERATING UNITS *(if any)*
Foreign - University of Barcelona, Sao Paolo, Barcelona, Spain

U.S. - NIMH - Clinical Neuroscience Branch

LAB/BRANCH
Diabetes Branch

SECTION
Receptor and Hormone Action Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Previously, insulin receptors could be demonstrated in membrane preparations of heads and bodies of chick embryo by 2 days of incubation. Binding of labeled insulin to brain and liver membranes from day 8 to 18 embryos increases with time of development. Recently, insulin-like growth factor binding was studied in chick embryo tissue. Using labeled insulin-like growth factor I and II (multiplication stimulating activity) and unlabeled homologous and heterologous peptides in competion binding assays, it appears that there are a specific insulin-like growth factor I but not insulin-like growth factor II receptors in the developing chick embryo. The growth factor binding pattern is different from insulin binding in several chick embryo tissues. Previously, we have demonstrated that insulin is present in chick embryos at day 2-3 as well as unfertilized eggs. To define insulin's role in early development anti insulin antibodies were injected into fertilized eggs and the effect of antibodies was studied. Antibody treated embryos had a higher rate of growth retardation and death by days 3-5 of embryogenesis; surviving embryos had delayed biochemical maturation compared to controls.

Insulin receptor and insulin-like growth factors (I and II) receptors structure studies have been extended in rat brain. The binding of labeled peptides to thin sections of frozen fresh rat brain was visualized with autoradiography. By several criteria including structure-activity relationship analysis, these brain peptide receptors were qualitatively indistinguishable from peptide receptors previously characterized on brain and other more typical target tissues and distinct from each other.

Each peptide exhibits its own distinctive binding pattern - i.e. each peptide binds to cytoarchetectonic structures. In addition, all three peptides demonstrate a high density of binding to choriod plexus.

Formerly Z01 AM 47025-02 DB

PHS 6040 (Rev. 1/84) GPO 814-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-DK-47026-02 DB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Tyrosine-Specific Protein Kinase Activity Associated with the Insulin Receptor

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	S. I. Taylor	Chief, Bio. & Mol. Path. Section	DB/NIDDK
Others:	N. Perrotti	V.A.	DB/NIDDK
	S. Brown-Phillips	Guest Worker	DB/NIDDK
	D. Accili	V. F.	DB/NIDDK
	S. DiPaolo	Guest Worker	DB/NIDDK
	R. Rees-Jones	Assist. Prof.	Columbia University
	Y. Zick	Assoc. Prof.	Weizmann Institute

COOPERATING UNITS *(if any)*
Columbia University, Dept. of Med., New York (Dr. R. Rees-Jones) U.S.
Weizmann Institute, Israel - Foreign

LAB/BRANCH
Diabetes Branch

SECTION
Biochemistry and Molecular Pathophysiology Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The insulin receptor possesses tyrosine-specific protein kinase activity. Multiple lines of evidence strongly support the hypothesis that this tyrosine kinase plays an important role in coupling insulin binding to insulin action. Nevertheless, the precise role of the tyrosine kinase activity has not been elucidated in detail. To address this question, we have attempted to identify physiologically important substrates for phosphorylation by the insulin receptor. We have identified one such substrate (pp120) which is a 120 kilodalton glycoprotein localized to the plasma membrane fraction of hepatocytes. In addition to serving as a substrate for the insulin receptor, pp120 can be phosphorylated by the hepatic epidermal growth factor receptor. Most importantly, it has been recently shown that insulin stimulates phosphorylation of tyrosine residues of pp120 in intact hepatoma cells.

Formerly Z01 AM 47026 01 DB

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 47027 - 01 DB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Use of SMS 201-995 in Hormone Secreting Tumors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	P. Gorden	Chief	DB, NIDDK
Others:	R. J. Comi	Medical Staff Fellow	DB, NIDDK
	N. Gesundheit	Medical Staff Fellow	MCNEB, NIDDK
	B. D. Weintraub	Chief	MCNEB, NIDDK

COOPERATING UNITS *(if any)*
Molecular Regulation and Neuroendocrinology Section, Molecular, Cellular, and Nutritional Endocrinology Branch

LAB/BRANCH
Diabetes Branch

SECTION
Clinical and Cellular Biology Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have studied the use of the long-acting somatostatin analogue, SMS 201-995, in acromegaly, TSH secreting pituitary tumors and glucagonomas. These studies have defined 1) an appropriate dose and schedule for control of TSH secretion from TSH secreting pituitary adenoma and its resultant hyperthyroidism, 2) an appropriate subgroup of acromegaly patients in whom this analogue, given thrice daily, controls GH hypersection, 3) the effects of the drug in glucagonoma syndrome in terms of control of glucagon hypersecretion and correction of hypoaminoacidemia. We are proceeding with long-term therapy of patients with the analogue to study effectiveness and side effects.

National Institute of Diabetes and Digestive and Kidney Diseases

I. Study of Immunology of Blood Cell Deficiencies

A. A Refined Mechanism for Attachment of Drug Antibody to Cells

We recently found that prototypic antibodies causing drug purpura are dependent only on the F(ab) region for reaction (see sections I Band, Ic). Because the Fc region is not involved, prior indications that antigen (drug)-antibody complexes are adsorbed by cells required reexamination. Key missing information has been accurrate measurement of potential reactions between antibody or platelets alone and drug, as well as antibody:drug ratios in complexes with cells. To assess Ka values for the various reactions by equilibrium dialysis we used purified (>95% drug-dependent IgG) and concentrated ($2x10^{-7}$ to $3x10^{-6}$M) antibodies from 5 patients with quinidine purpura, ^{3}H-labeled drug, and platelets or their purified membranes with receptor capacity determined by RIA assay of drug-dependent IgG at saturation. Drug reaction with antibody was measureable only at highest antibody concentration. Based on 14 determinations, the Ka was a mean of $5.7x10^{4}$ with variations from $4x10^{4}$ to 10^{5} not influenced by drug concentration over the range of $5x10^{-9}$ to $5x10^{-6}$M. When platelets were saturated with antibody at $3.9x10^{-6}$M drug concentration the highest KJa between antibody and drug if the complex was $2x10^{8}$ and the lowest was $6x10^{5}$. Antibody:drug molar ratio in these complexes, based on the RIA value for adsorbed antibody and the difference in concentration of drug on equilibrium dialysis when platelets alone, compared to platelets plus antibody were opposite drug, was ~1:2 for the highest affinity complex, and 1 to 1.5 for the lower affinity complexes. A model that fits these various findings is: 1) Antibody associates specifically with drug in a weak haptenic reaction; cell interaction with drug is nonspecific but may be catalytic by generating a high local concentration of reactants at the liquid-solid interface. 2) Conformational change in Fab induced by drug binding results in complementarity between Fab and cell receptor. 3) Steric rearrangement of both Fab and cell receptor maximize complementarity and affinity.

B. A Tolmetin-Dependent Antibody Causing Severe Intravascular Hemolysis Binds to Erythrocyte Band 3 and Requires Only the $F(ab)_2$ to React

Serum from a patient with acute intravascular hemolysis was found to contain drug dependent (dd) IgM that caused C' fixation and agglutination, and dd IgG that could be detected only by specific in direct antiglobulun testing.

In the present work we found that the receptor for the dd-reaction was on all cells of a standard blood bank panel as well as on O_{Bombay}, Rh null, K_o, Jk (a-b-) and adult I-negative cells. Reactions of cells from cord blood were quantitatively the same as adult RBC's. Thus, none of these blood group antigens was the receptor for the Tolmetin Ab.

Solubilized RBC membranes were transferred by Western blot and incubated with dd-antibody followed by by a second incubation with either ^{125}I-protein A or ^{125}I-anti IgG, all in the presence of drug. The dd Ab receptor could not be identified this way. However, ^{125}I-labelled RBC membranes reacted with dd Ab, solubilized, precipitated by staph. protein A, eluted, reduced and electrophoresed in SDS-PAGE, produced an autoradiographic line primarily at the level of band 3. An antibody specific for band 3 produced the same pattern as dd Ab by this technique.

^{125}I-F(ab)$_2$ fragments, prepared by pepsin digestion of DEAE chromatographed IgG and chloramine T labelling, bound to RBC's in drug-dependent fashion the same as dd IgG. Proof that the bound material was ddF(ab)$_2$ and not a possible contaminant was obtained by eluting bound dd-^{125}I-labelled protein from RBC's and performing SDS-PAGE followed by autoradiography. More than 90% of the eluted protein had a molecular weight of 100,000. The apparent association constant of the dd ^{125}I F(ab)$_2$ binding was approximately 2 x $10^8 M^{-1}$, and equal to that of intact IgG from which it was made.

These findings are similar to those for the antibody of quinidine purpura in which the F(ab)$_2$ moiety of dd IgG combines with a major membrane glycoprotein (see section Ic).

C. Binding of Quinine- and Quinidine-Dependent Drug Antibodies to Platelets is Mediated by the Fab Domain of the IgG and is not Fc-Dependent

Characteristics of the binding of drug-dependent antiplatelet antibodies to platelet membranes have been stuydied utilizing purified IgG, F(ab')$_2$, Fab, and Fc fragments from each of three patients with cinchona alkaloid-induced thrombocytopenia. Direct binding of each component to purified platelet membranes in the presence and absence of drug was quantitated by radioimmunoassay. A single batch of purified platelet membranes was used throughout to assure comparable results in the various experiments. The number of antibody molecules bound per platelet equivalent of purified platelet membranes at apparent saturation varied from approximately 20,000 to 50,000 for three antibodies and the affinity of the reactions was in the range of 10^7 to $10^8 M^{-1}$. The ability of IgG fragments to bind to platelet membranes drug-dependently and to competitively inhibit drug-dependent binding of radiolabelled intact IgG was quantitatively measured. Results of such competitive inhibition were corrobor-

ated using complement fixation as the indicator of drug-antibody binding for the same mixtures of reagents. The $F(ab')_2$ fragments of the three antibodies bound to platelet membranes in a drug-dependent fashion, competed at a 1.6-3:1 molar ratio with IgG binding in complement fixation assays and ~5:1 molar ratio by radioimmunoassay (RIA). Drug-mediated Fab binding was further demonstrated by the SDS-PAGE pattern of eluates prepared from platelets exposed to radiolabelled $F(ab')_2$ fragments in the presence and absence of drug. Fc fragments showed no inhibitory effects upon either drug-dependent radiolabelled IgG binding or IgG-mediated complement fixation. Fc fragments bound directly to platelet membranes, but the extent of binding was the same in either the presence or absence of drug. We conclude that the Fab rather than the Fc domain of the IgG supports drug-dependent attachment to the platelet surface. The ternary complex formed by interacitons among drug, the membrane receptor, and the Fab domain of the IgG may be initiated by binary reactions between drug and either Fab domain, the cell membrane, or both.

D. An Apparent "New" Platelet Antigen Identified by an Antibody Causing Post Transfusion Purpura

A patient who responded to plasma exchange for post transfusion pupura was found to be Pl^{A1}-positive and have a c'-fixing antibody in his serum (ser-Ni) that reacted with platelets from 63 of 95 normal donors and did not react with lymphocytes. Triton solubilized platelets of the same panel of 95 donors were subjected to SDS-PAGE, transferred to nitrocellulose, incubated with an eluate from ser-Ni using platelets of a strong reactor, and exposed to ^{125}I-staph. protein A. Positive reactors yielded single bands by autoradiography at a MW of 135,000. Purified GPIIb run along with the panel produced a band at the same level but there was no reaction with GPIIIa or GPI complex. By cellulose transfer, 82 of the 95 donors were positive (87%), giving a gene frequency for the recognized antigen of 0.63. This gene frequency differs significantly from that reported for Lek^a of 0.86 based on 98% positive reactors. Bak^a positive reactors are 90.76%. However, in the cellulose transfer test, of 12 negative reactors with ser-Ni, 2 were strongly positive with Bak^a antiserum. Moreover, immunoprecipitation studies using purified ^{125}I-labeled GPIIb and anti-Bak^a serum (limited in scope by available quantities of antiserum) gave no indication of binding activityabove that of normal control serum whereas ser-Ni reacted strongly at high dilutions.

Reactions dependent on C' fix may fail to give accurate phenotype frequencies with certain antisera but the immunoblot technique appears to be an unusually sensitive and reliable one for detecting platelet antigens. If Bak^a and Lek^a are similar antigens (as some report) then the one defined by ser-Ni is "new". If Bak^a is distinct from Lek^a, there is a possibility that the anti-Bak^a we had available to us was as too weak for full characterization and the antigen we defined may prove to be the same as Bak^a.

E. Internal Platelet Receptors for IgG, IgM and IgA Reactive with all Normal Sera.

Recently, several reports have suggested that autoantibodies formed in association with drug-, allo-, or anti-viral, antibodies are responsible for thrombocytopenia. Indication of autoantibody formation is appearance of lines of reaction in Western blots of solubilized platelets when patients' sera are used to develop the blots, followed by labeled anti-IgG to identify proteins that are apparently targets of antibodies. However, these studies have not been adequately controlled by patterns that normal sera may form with normal platelet proteins on Western blots.

Normal background pattern produced when Western blots of solubilized platelets are developed with sera were studied. Fifty normal sera all gave a band at 90K M_r with anti-IgG, -IgM, or -IgA when incubated with blots of nonreduced autologous (10) or homologous (50) platelets and developed with the biotin-avidin-peroxidase system. Titers (dilution^{-1}) ranged from 4-1280 for IgG, 5-80 for IgM, and 20-640 for IgA. When 50 sera were exposed to the blots of one donor's platelets, or a single serum was exposed to blots of 50 platelets, additional discrete bands at M_r 140K, 115K, 95K, 85K, 80K, 65K, 60K, and 50K were obtained with anti-IgG, -iGM or -IgA with frequencies varying from 1-55%. Lower M_r bands were not defined on the 7% gels. The number of bands per blot varied with respect to both the platelet blotted and n ormal serum used, ranging from 1 to 9, with a median of 4 for anti-IgG, 3 for -IgM and 1 for IgA. Titers varied for different bands with a single serum, but none exceeded that for the 90K band. Titers of 6 donors did not change significantly in serial serum samples over several years. In all reactions, IgG and its $F(ab')_2$ were interchangeable. The 90K M_r band appears distinct from $GPIII_a$ because it was present in thrombasthenic platelets lacking $GPII_b/III_a$, was not obtained with membranes purified by glycerol lysis or with purified $GPIII_a$, and maintained a 90K M_r under reduced conditions. The 90K M_r band was present only in the membranous fraction of sonicated, freeze-thawed platelets or thrombin-treated platelets. Other bands were distributed in either the soluble or membranous fractions of mechanically lysed platelets. Fresh platelets or the equivalent in purified membranes used to adsorb serum ($2x10^9$/ml) did not diminish titer for the 90K band; but stored or freeze-thawed platelets decreased titers, and platelets solubilized in 2% SDS before incubation in serum eliminated titers. Thus, the 90K and other receptors for IgG, IgM, and IgA appear to be internal, non-plasma membrane components that may account in part for elevations of platelet-associated nonspecific immunoglobulins in diseases of platelet injury. The lines that normal immunoglobulins form with these receptors may be misinterpreted as reactions of pathogenic asutoantibodies in platelet disorders due to other causes.

II. A Study of Blood Coagulation and Diseases of Hemorrhage and Thrombosis

A. The Association of the Human Platelet-Specific Alloantigen, Pl^{E1}, with the Alpha Subunit of Glycoprotein Ib

Two human platelet alloantigen systems, Pl^A and Bak (Lek) have been localized to components of the highly immunogenic GPIIb-IIIa complex. We now provide evidence that a third alloantigen system, Pl^E, is associated with a different membrane glycoprotein, namely, the alpha subunit of GPIb. By ^{51}Cr release, platelets from two of three patients with the Bernard-Soulier syndrome (BSS) responded subnormally to anti-Pl^{E1} antibody. The apparently normal response of platelets from the last BSS patient was found to result from reaction of his HLA-A2 positive platelets with high titer anti-HLA-A2 present in the anti-Pl^{E1} plasma. The nonreactive BSS patients were both HLA-A2 negative. These preliminary results raised the possibility that the Pl^{E1} alloantigen is associated with the GPIb complex known to be absent from BSS platelets. To confirm this possibility, an ELISA was employed in which the purified GPIb complex and glycocalicin were used as solid-phase antigen. Anti-Pl^{E1} antibody bound specifically to both the GPIb complex and glycocalicin. ^3H-labeled platelet membrane glycoproteins with apparent membrane glycoproteins with apparent molecular weights of 130K, 25K, and 21K (under reduced conditions) corresponding to GPIbα, GPIbβ, and GPIX were specifically immunoprecipitated by anti-Pl^{E1} plasma. Under nonreduced conditions, protein bands with apparent molecular weights of 170K and 22K, corresponding to GPIb and GPIX, were detected. Finally anti-Pl^{E1} completely inhibited ristocetin-induced platelet agglutination at a titer of 1:16. These results indicate that the PL^{E1} alloantigen is localized to a region of GPIb alpha that is also conserved in its proteolytic derivative, glycocalicin.

B. An IgM Causing Spurious Thrombocytopenia

We originally described the occurrence of nonspecific platelet agglutination *in vitro* in blood samples collected in a variety of anticoagulants. This has been a cause of falsely low platelet counts, because capillary blood samples taken from the same patients and diluted before agglutination occurs have normal platelet content. Spurious thrombocytopenia may lead to erroneous diagnosis of ITP or drug-induced thrombocytopenia. Although many patients whose blood contains an agglutinin have sytemic disease, many do not; the nature and cause of agglutinin has been obscure.

We have found in a patient with rheumatoid arthritis an unusually potent *in vitro* agglutinin causing pseudothrombocytopenia. The factor was an IgM, $F(ab')_2$-mediated, reactive only at low Ca^{++} concentrations with a glycoprotein in platelet membranes and best at temperatures below 30^o, identified as GPIIb. We have

recently identified an alloantibody with specificity against an epitope that also is on GPIIb. As demonstrated by lack of competition between the IgG alloantibody and IgM agglutinin for platelets or for GPIIb on Western blots, the different immunoglobulins react with separate epitopes on the GPIIb molecule. A number of antibodies responsible for ITP also react with GPIIb but are IgG and do not have binding the characteristics of the pseudo-agglutinin. Although pseudoagglutinins have not been implicated in abnormal platelet function or in thrombocytopenia it is possible that, under some circumstances for example during heparin anticoagulation--the agglutinins may cause platelet destruction in vivo and their reactions may differ only in degree from immunoglobulins causing ITP.

C. Clinical Cases of Special Interest

1. A New Syndrome of Congenital Thrombocytopenic Purpura

A child with the diagnosis of ITP from birth was found by us to have instead a group of findings not previously described. The patient was thrombocytopenic but platelet survival and organ localization of transfused platelets was normal. His platelets were approximately 1/5 normal size but the platelet membranes appeared to have normal proportions of qualitatively normal glycoproteins. By electron-microscopy, the small platelets had fewer cytoplasmic organelles per platelets than normal, but ultrastructure otherwise appeared normal. Aggregation in response to cell stimuli was suppressed, suggesting that small size per se interferes with inter-platelet reaction. The findings resembled most closely those of the Wiskott-Aldrich syndrome but our patient does not have eczema, immune deficiency, or multiple infections associated with this syndrome. Our patient appears to have an unusual platelet production defect which we will further define by studies of cultured megakaryocytes function.

2. Unusual Cases of Drug-induced Thrombocytopenic Purpura and Hemolytic Anemia

Of patients referred with diagnoses of ITP or autoimmune hemolytic anemia in the past 3 years we have had four in whom the disorder was self-inflicted. Each had had an initial spontaneous occurrence of drug-induced hemorrhage or hemolysis that was undiagnosed; and subsequently, for secondary gain, initiated these life-threatening occurrences by self medication. Their disorders mimicked intermittant, unusually severe, acute ITP and autoimmune anemia. We were able to diagnose a drug-induced immune disorder in each case because of sensitive screening techniques we have developed for detecting drug-antibodies in patients with unusual symptomatology. The antibodies found in these cases have been used in basic studies of antigen-antibody reactions with cells (see studies IA and B).

These patients represent the first reportable instances of self-inflicted immunologically-induced blood cell deficiency states.

3. New Methods for Diagnosing and Treating Isoimmune Neonatal Thrombocytopenia (INT)

In order to diagnose the many suspected cases of INT referred for study and to prognosticate the likelihood of the disorder recurring we have developed sensitive methods for measuring maternal antibodies against platelet membrane antigens, including techniques of immunoprecipitation, ELISA, immunofluorescence, and Western blotting with labeled antiglobulins. With these methods of measurement we have been able to predict likelihood and severity of recurrence in 12 of 12 instances. We have experimentally evaluated the use of intravenous gamma globulin given to mothers antenatally to effect either transfer of therapeutic IgG to infants or to competitively block transfer of maternal alloantibodies across the placenta. In 2 cases this type of therapy appeared to be highly successful. Intravenous gamma globulin has also proved to be successful therapy for infants with INT.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 51,000-28 CHB
formerly:
Z01 AM 51,000-27 CHB

PERIOD COVERED
October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Study of Immunology of Blood Cell Deficiencies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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	Charles Jones	Chemist	CHB,NIDDK
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COOPERATING UNITS *(If any)* Walter Reed Army Institute of Research (B.Alving); Univ. of Texas Medical School at Houston (A.W.Bracey); NYU Med. Ctr., Dept. of Med. (D. Zucker-Franklin); Dept. of Med., Christ Hospital, Chicago (J.Jordan) Tulane Univ., Dept. of Med. (C. Leissinger)

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2-1/2	1-1/2	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The immunologic disorders, idiopathic thrombocytopenic purpura (ITP), neonatal (INT)-post-transfusion (PTP) and drug-purpura account for the majority of clinical thrombocytopenias of immune origin. The antibody reactions involved in these disorders are relevant to understanding autoimmunity,histocompatibility,malignant surveillance, isoimmune reactions, disorders due to antigen-antibody complexes and cellular immune injury generally. We have developed more precise and sensitive assays for cell-associated IgG,IgM,IgA and albumin than heretofore available and found that immune injury by specific antibody causes platelets to associate with all classes of immunoglobulins and with other serum proteins. By electronmicroscopy this association appears to result from trapping of plasma protein by resealed cytoplasmic-free platelet membranes. An animal model for ITP was developed that duplicated the cellular and immunologic abnormalities of this disease. Three additional cases of PTP were found to be due to isoantibodies against previously unrecognized platelet antigen specificities.We have extensively determined the molecular characteristics of one of these determinants. Nitrocellulose transfer techniques and a precipitin test utilizing labelled platelet proteins were used to detect antiplatelet antibodies of INT and PTP more sensitively, specifically and easily than previously possible. Platelet membrane receptor glycoproteins were purified as reactants in diagnostic tests for isoantibodies,and drug antibodies. We found that the Fab portion of drug antibodies is responsible for their attachment to platelet and red cell surfaces, correcting erroneous reports that the Fc portion is responsible. Association constants of drug-antibody reactions with platelets and red cells have been established by saturation kinetics and equilibrium dialysis, and the red cell receptor for a tolmetin-dependent hemolytic antibody was found to be the band 3 anion channel protein. Utilizing antibodies from patients that reacted specifically with platelet glycoprotein I,IIb or IIIa, bone marrow megakarycyte progenitors were detected by immunofluorescence before cells could be differentiated morphologically.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 51,001-28 CHB
formerly
Z01 AM 51,001-27 CHB

PERIOD COVERED
October 1, 1985 through Septmber 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Study of Blood Coagulation and Diseases of Hemorrhage and Thrombosis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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LAB/BRANCH
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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2-1/2	1-1/2	1

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☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The physiology of platelet secretion has many features in common with the secretory physiology of endocrine and neuronal cells; and a number of the biogenic amines synthesized, stored and secreted by these different cell types are similar. Platelet membrane glycoproteins (GP) appear to be major factors determining cell-cell recognition, and secretion. We have identified a new epitope of GPIb that acts as a receptor for von Willebrand'sfactor which controls platelet endothelial interaction but does not interfere with platelet seretion or reaction with drug antibodies that also interact with GPIb. In recent years thrombocytopenic states associated with acquired immune deficiency syndrome, post-transfusion purpura, and viral exanthens have been attributed in part to apparent autoantibodies identified by anti-globulin reactions with IgG, IgA, or IgM attached to Western blots of solubilized platelets. We have found a high frequency of identical anti-immunoglobulin bands in normal sera, which appear to be internal(non-plasma membrane)Ig receptors. These receptors in other cells may account for similar apparent autoantibody reactions and in platelets for increases in platelet associated immunoglobulin in diseases of platelet destruction such as ITP.

Early events in megakaryopoiesis involving stem cell differentiation, are poorly understood because megakaryocyte progenitors cannot be identified by conventional microscopic techniques. We have found that human leukemic cell lines(HEL and K562), which differentiate into cells that have surface marker glycoproteins similar to platelets also express a growth promoting factor that is similar to the transforming growth factor b-like protein(s) (TGF) from platelets. These conclusions are based on comparisons of platelet TGF and TGF from HEL and K562 by their characteristics on Bio-Gel columns,anchorage-independent growth promoting activity, molecular weights on SDS-PAGE, and amino acid composition. Growth factors appear to play a key role in endothelial repair and homeostasis and are of special importance in diseases of abnormal endothelium such as arteriosclerosis.

ANNUAL REPORT SUMMARY OF THE GENETICS AND BIOCHEMISTRY BRANCH

NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

Biochemical Genetics Section

Dr. Myerowitz and collaborators have continued their studies of the DNA coding region for human alpha-chain of beta-hexosaminidase A (a deficiency of which is responsible for Tay-Sachs disease). During the past year they have studied the genetic lesions present in the alpha-chain of Tay-Sachs patients of non-Jewish and Ashkenazi Jewish origins. They have found that the mutation is different in these two groups of patients. The gene appears intact in the Jewish patients. In contrast, the alpha-chain gene of the non-Jewish French Canadian patients has a 5' deletion of approximately 7 kilobases.

Dr. Proia and collaborators have isolated human genomic clones covering the entire gene encoding for the alpha chain of beta-hexosaminidase A. The alpha chain gene spans 35 kilobases of DNA and is split into 14 exons. They found that the position of introns matches potential protein processing sites, indicating a possible correlation of gene structure with protein structure. They have also isolated cDNA and genomic clones encoding the beta chain of beta-hexosaminidase A. Sequence analysis of these clones indicates extensive homology with the alpha chain with significant differences at the amino-termini of the two polypeptide subunits.

Dr. Robbins and her colleagues have continued their approach to dissecting the processes of endocytosis, glycoprotein biosynthesis and sorting through their isolation and analysis of mutants. They have found that the majority of CHO cell endocytosis mutants represent only two complementation groups. They have recently isolated endocytosis mutants of Ltk^- cells; the phenotypes of these mutants differ markedly from the CHO mutants.

In their study of glycoprotein biosynthesis they have extensively characterized a new glycosylation mutant. The phenotype of this mutant is consistent with a defect in translocation of the lipid-linked Man_5 intermediate from the cytoplasmic to the luminal face of the ER membrane.

Finally, they have examined the cellular sorting of acid hydrolases by using their temperature-sensitive CHO cell endocytosis mutants. Acid hydrolases are transported to lysomes by both an endocytic and an intracellular pathway; the acidic endosome has been proposed as the junction of these pathways, with receptor redistributing from this compartment to both pathways. Their data, however, suggests that these pathways meet only after segregation of receptor from the acid hydrolases.

Molecular Genetics Section

Dr. Ackerman and collaborators have continued their work on an oocyte specific gene product called OAX RNA for oocyte activated in Xenopus. This RNA is 181 nucleotides long, present in approximately 10,000 copies/oocyte and is in a cytoplasmic complex of approximately 50S size. This RNA is not found in adult somatic tissue.

In addition, they investigated the presence of homologies to the human or rodent LINE-1 or LI family of repetitive DNA in Xenopus. Using the 3' end of a mouse L1 probe they have found RNA expressed in Xenopus Oocytes, and embryonic and adult tissues.

Dr. Camerini-Otero and his colleagues$Fhave continued their studies of genetic recombination in high eukaryotes. They have partially purified and characterized a recombinase activity from human B cell lymphoblasts. Stoichiometric amounts of this recombinase carry out a strand transfer reaction between linear duplex DNA and a homologous circular single-strand DNA. The product of this strand transfer reaction is a joint molecule composed of a single-strand joined to one end of the linear duplex by a region of DNA heteroduplex. Formation of these heteroduplexes is accompanied by strand displacement. This is the first demonstration of a strand transfer activity from a high eukaryote. Work is now in progress to purify this protein to homogeneity and to clone the gene encoding it.

In addition, they have been able to partially purify and characterize a similar protein from early embryos of Drosophila melanogaster. Work is now in progress to identify a recombinase deficient mutant among the recombination defective mutants of Drosophila.

Finally, Dr. Camerini-Otero's colleagues are investigating the role of this B cell lymphoblast-derived recombinase in immunoglobulin rearrangements.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 52008-07 GBB
formerly
Z01 AM 52008-06 GBB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Gene Expression and Human Genetics

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	P. Pittman	Guest Researcher	GBB, NIDDK
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8.0	6.6	1.4

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☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have partially purified and characterized a human recombinase activity from RPMI 1788 B lymphoblasts. Stoichiometric amounts of recombinase carry out a strand transfer reaction between linear duplex DNA and a homologous circular single-strand DNA. The product of this strand transfer reaction is a joint molecule composed of a single-strand circle joined to one end of the linear duplex by a region of DNA heteroduplex at least 150 bp long. Formation of DNA heteroduplexes is accompanied by strand displacement. Strand invasion initiates at the ends of the linear duplex. Finally, strand displacement by human recombinase exhibits polarity and proceeds in a 3' to 5' direction. This is the first demonstration of a strand transfer activity from a high eukaryote.

More recently we have pursued the purification through several columns to the point where most contaminating enzymatic activities have been removed. In addition, we have partially characterized and purified a similar protein from early embryos of Drosophila melanogaster.

Finally, we have investigated the role of this lymphoblast-derived recombinase in immunoglobulin rearrangements. The proper double-strand and single-strand substrates were prepared from synthetic oligonucleotides containing the D and J recombination signal sequences. Preliminary data indicate that these D and J regions can participate in a strand exchange or transfer reaction in vitro.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 52009-07 GBB
formerly
Z01 AM 52009-06 GBB

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Endocytosis, Secretion, and Compartmentalization in Mutant CHO Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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Genetics and Biochemistry Branch

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Biochemical Genetics Section

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NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.2	4.0	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our approach to dissecting the processes of endocytosis, glycoprotein biosynthesis and sorting is through isolation and analysis of mutants. We have found that the majority of CHO cell endocytosis mutants, whether non-conditional or temperature-sensitive, isolated by ourselves and others using disparate strategies, represent only two complementation groups. Using our standard isolation procedures we have recently isolated endocytosis mutants of LTk- cells; phenotypes of these mutants differ markedly from the CHO mutants.

Acid hydrolases are transported to lysosomes by both an endocytic and an intracellular pathway; the acidic endosome has been proposed as the junction of these pathways, with receptor redistributing from this compartment to both pathways. However, on return of temperature-sensitive CHO cell endocytosis mutants to the permissive temperature, we observe drastically different rates for restoration of surface receptor activity versus intracellular transport. Our data suggest that these pathways meet only after segregation of receptor from the acid hydrolases.

We have characterized a glycosylation mutant that 1. transfers oligosaccharide from lipid to protein in decreased amounts; 2. fails to elongate the lipid-linked GlcNAc2Man5 intermediate in vivo; 3. accumulates the sugar donor mannosyl phosphoryl dolichol in elevated amounts; 4. elongates the Man5 in vitro in the presence of detergent. This phenotype is consistent with a defect in translocation of the lipid-linked Man5 intermediate from the cytoplasmic to the luminal face of the ER membrane.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 52011-02 GBB
formerly
Z01 AM 52011-01 GBB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Oocyte Specific Genes in Amphibian Embryogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Eric Ackerman	Senior Staff Fellow	GBB, NIDDK
Others:	Kuldeep K. Bhatia	Visiting Fellow	GBB, NIDDK

COOPERATING UNITS *(if any)*
DAAD - German Academic Exchange Service and the Free University of Berlin (Norbert Ulbrich and Roland K. Hartmann).
Medical Genetics Fund (Emily D. Cox)

LAB/BRANCH
Genetics and Biochemistry Branch

SECTION
Molecular Genetics Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	1.5	0.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

I. No gene transcription occurs in fertilized eggs of the amphibian Xenopus until the mid-blastula transition, (approximately 4000 cells). Therefore all of the appropriate gene products necessary for early development must be stored in the oocyte or egg prior to fertilization. In order to understand the molecular embryology of early amphibian development, we must obtain genes whose products are expressed only in oocytes. We have been working on an oocyte specific gene product called OAX RNA for oocyte activated in Xenopus. This RNA is 181 nucleotides long, present approximately in 10,000 copies/oocyte and is in a cytoplasmic complex of approximately 50S size. This RNA is not found in adult somatic tissue.

II. The Aspergillus toxin alpha-sarcin produces a precise cut near the 3'-end of 28S ribosomal RNA in vitro only if the ribosomes are pre-treated with puromycin and EDTA. Alpha-sarcin can also behave as a general nuclease in vitro under appropriate conditions. In order to investigate alpha-sarcin's in vivo activity, we injected it into living Xenopus oocytes and analyzed the resulting RNA.

III. The human and rodent LINE-1 or L1 family of repetitive DNA is present more than 10,000 times per haploid genome. The L1 family has long open reading frames and transcripts present in various types of cells. Using a probe from the 3'-end of a mouse L1 gene, we looked for the presence of RNA transcripts in Xenopus oocytes, embryonic and adult tissues.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 52012-02 GBB
Formerly
Z01 AM 52012-01 GBB

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure-Function Relationships of Lysosomal Enzymes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Richard L. Proia	Senior Staff Fellow	GBB, NIDDK
Others:	Emilia Soravia	Visiting Fellow	GBB, NIDDK
	Sybille Sonderfeld-Fresco	Guest Worker	GBB, NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Genetics and Biochemistry Branch

SECTION

Biochemical Genetics Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.4	2.3	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Beta-hexosaminidase has become a paradigm for the understanding of lysosomal enzyme biology. In order to gain further insight into the hexosamindase system, we have isolated human genomic clones covering the entire gene encoding the alpha chain of the enzyme. These clones have been characterized by restriction mapping, Southern blotting and DNA sequencing. The alpha chain gene spans 35 kilobases of DNA and is split into 14 exons. The position of introns matches potential protein processing sites, indicating a correlation of gene structure with protein structure. The promoter region of this gene contains conventional "TATA" and "CAAT" motifs. We have also isolated cDNA and genomic clones encoding the beta chain of hexosaminidase. Sequence analysis of these clones indicate extensive structural homology with the alpha chain with significant differences at the amino-termini of the two subunits.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 52013-02 GBB
formerly
Z01 AM 52013-01 GBB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Genetic Lesion of Tay-Sachs Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. Myerowitz	Senior Staff Fellow	GBB, NIDDK
Others:	N. Hogikyan	Medical Student, Univ. of Michigan	

COOPERATING UNITS *(if any)*

LAB/BRANCH
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SECTION
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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.2	1.0	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Tay-Sachs disease is an inherited disorder caused by mutation in the alpha-chain of beta-hexosaminidase A, a lysosomal enzyme composed of two polypeptides designated the alpha and beta chains. The disease is heterogeneous displaying a wide range of severity and age of onset. An early onset and fatal form of the disorder referred to as "classic" Tay-Sachs disease has a ten fold higher gene frequency among the Ashkenazi Jews and a population of non-Jewish French Canadians located in Eastern Quebec.

We previously isolated and characterized a cDNA clone containing the entire coding sequence for the alpha-chain of beta-hexosaminidase. During the past year we have utilized this clone to study the genetic lesions present in the alpha-chain gene of Tay-Sachs patients of Ashkenazi Jewish and non-Jewish French Canadian origin. We have found that the mutation differs in these two groups. The alpha-chain gene of the Ashkenazi Jewish patient appears intact suggesting a subtle genetic lesion. In contrast, the alpha-chain gene of the non-Jewish French Canadian patients has a 5' deletion of approximately 7.4 kilobases.

PHS 6040 (Rev. 1/84)

GPO 914-918

ANNUAL REPORT OF THE DIGESTIVE DISEASES BRANCH

NATIONAL INSTITUTE OF DIABITES AND DIGESTIVE AND KIDNEY DISEASES

SUMMARY OF BRANCH ACTIVITIES

The Digestive Diseases Branch has two sections (Section on Gastroenterology and the Liver Diseases Section). The Liver Diseases Section has 2 senior physicians and 3 medical staff fellows; the Section on Gastroenterology has 3 senior physicians and 5 medical staff fellows. The Digestive Diseases Branch also has 5-10 guest investigators fron other laboratories.

Detailed summaries of the activities of each section precede the individual project reports. Both sections are engaged in investigations of basic biologic processes (e.g., hormone action, membrane transport, cellular and humoral immunology) and are attempting to apply this information to understand the pathophysiology of various disorders involving the liver and gastrointestinal tract. Both sections are also involved in attempts to improve therapy of clinical disorders such as neoplasms associated with overproduction of gastrointestinal hormones, hepatitis and fulminent hepatic failure.

Section on Gastroenterology

The Section on Gastroenterology is currently following approximately 100 patients with Zollinger-Ellison syndrome (ZES, gastrin-producing neoplasm, hypergastrinemia and increased secretion of gastric acid). All patients are currently being treated with oral medication that inhibits gastric acid secretion.

Although histamine H_2-receptor antagonists are effective inhibitors of gastric acid secretion in patients with Zollinger-Ellison syndrome, these agents must be taken in large doses and at frequent intervals. Omeprazole a new antisecretory agent that inhibits gastric H^+,K^+-ATPase was tested for therapeutic efficacy in patients with Zollinger-Ellison syndrome. A single dose of omeprazole inhibited gastric acid secretion for more than 48 hours in patients with Zollinger-Ellison syndrome. In 90% of patients with Zollinger-Ellison syndrome, gastric acid secretion could be adequately inhibited by a single daily dose of omeprazole. Omeprazole was free of detectable toxicity during three years of therapy. Because of its long duration of action, omeprazole offers an advance in convenient medical therapy for Zollinger-Ellison syndrome compared with histamine H_2-receptor antagonists.

Famotidine, a new, potent, long-acting histamine H_2-receptor antagonist was compared to cimetidine and ranitidine in patients with Zollinger-Ellison syndrome. Equally potent doses of the three drugs had similar onsets of action, but the duration of action of famotidine was 30% longer than the duration of action of either ranitdine or cimetidine. Famotidine was 9-times more potent than ranitidine and 32-times more potent than cimetidine. Patients have been treated for up to 30 months with good control of gastric acid secretion with no evidence of biochemical or hematologic toxicity.

Pancreatic cholera is characterized by severe watery diarrhea, hypokalemia and acidosis, and in most cases is due to a non-β-islet-cell tumor of the pancreas that secretes vasoactive intestinal peptide and peptide histidine isoleucine. When such tumors are localized surgical resection may produce a cure, and in patients with metastatic disease streptozotocin may reduce the tumor's size and control symptoms. A variety of agents - corticosteroids, lithium, metoclopramide, clonidine, trifluoperazine and indomethacin - have been reported to reduce or abolish diarrhea in various patients with pancreatic cholera. Because continuous infusion of somatostatin has been reported to reduce diarrhea in a patient with pancreatic cholera, we tested the effect of a new, long-acting analogue of somatostatin (SMS 201-995) in a patient with pancreatic cholera who was refractory to all other forms of therapy. In this patient SMS 201-995, given twice a day as a subcutaneous injection, eliminated diarrhea and allowed correction of dehydration, hypokalemia and acidosis.

It is known that gastrinomas may produce other peptides besides gastrin. During this past year we have established that in approximately 5 percent of patients with sporadic gastrinoma the tumor also secretes significant amounts of ACTH giving rise to a florid form of Cushing's syndrome. In contrast to patients with sporadic gastrinoma, 20% of patients with gastrinoma and MEN-I have Cushing's syndrome resulting from pituitary overproduction of ACTH.

Gastric chief cells from guinea pig stomach possess cell membrane receptors for VIP and secretin. In fact, chief cells possess four functionally distinct classes of receptors that interact with VIP and secretin. Two of these classes lead to increased cellular cyclic AMP and stimulation of pepsinogen secretion, whereas the other two classes produce no detectable change in cell function and may represent degradation sites for the peptides.

Several years ago we showed that pancreatic acinar cells possess a cyclic AMP-dependent protein kinase, activation of which mediates the actions of secretagogues that increase cellular cyclic AMP (e.g., secretin, vasoactive intestinal peptide, peptide histidine isoleucine, Gila monster venom, cholera toxin). During this past year we found that pancreatic acinar cells possess a calcium-activated, phospholipid-dependent protein kinase (protein kinase C), activation of which mediates the actions of secretagogues that mobilize cellular calcium (e.g., cholinergic agents, cholecystokinin, gastrin, bombesin, litorin, substance P, physalaemin). For agents such as the phorbol esters which directly activate protein kinase C, there is a close correlation between the potency with which the phorbol ester activates the enzyme and the potency with which the phorbol ester stimulates pancreatic secretion.

There are three classes of CCK-receptor antagonists: derivatives of cyclic nucleotides, derivatives of amino acids and fragments and analogues of the C-terminal region of CCK. Previously we showed that N-acyl derivatives of tryptophan were specific CCK receptor antagonists and of these, N-carbobenzoxy tryptophan was the most potent antagonist. During this past year we examined various carbobenzoxy amino acids to explore the role of the amino acid side chain in influencing inhibitory potency. Of the carbobenzoxy amino acids tested, carbobenzoxy cystine was the most potent CCK receptor antagonist. The

hydrophobicity of the amino acid side chain is a primary determinant of the inhibitory potency of the carbobenzoxy amino acids.

During this past year we found that N-terminal fragments of CCK-26-33 can funcion as specific, reversible, competitive CCK receptor antagonists. In particular, CCK-26-32, CCK-26-31 and CCK-26-30 were CCK receptor antagonists, whereas CCK-26-29, at concentrations as high as 100 μM had no CCK receptor antagonist activity. Various structural modifications affected antagonist activity differently depending on the length of the peptide. For example, removing the C-terminal amide from CCK-26-31-NH_2 caused a 10-fold decrease in inhibitory potency, whereas removing the C-terminal amide from CCK-26-30-NH_2 did not alter the inhibitory potency of the peptide. Moreover, removing the sulfate ester from the tyrosine in position 27 of CCK-26-31 did not alter the inhibitory potency of the peptide, whereas removing the sulfate ester from the tyrosine in position 27 of CCK-26-30 caused a 5-fold decrease in inhibitory potency.

In dispersed acini prepared from mouse pancreas, bombesin and structurally related peptides cause significant stimulation of enzyme secretion. During this past year we made the surprising observation that when acini are first incubated with bombesin or a related peptide and then washed and reincubated, enzyme secretion during the second incubation is 30 percent greater than when bombesin or a related peptide is added directly to the incubation. The basis for this phenomenon is not clear; however, its elucidation should provide insight into the regulation of receptor-effector coupling in mouse pancreatic acinar cells.

Five years ago we showed that proglumide was a specific CCK receptor antagonist. During this past year we have examined various structural analogues of proglumide and have found that these analogues are approximately 1,000-times more potent than proglumide at inhibiting the interaction of CCK with its cell surface receptors on various target tissues.

The Liver Diseases Section is currently conducting seven principal studies.

I. Studies Relating to the Pathogenesis of Hepatic Encephalopathy

The patterns of visual evoked responses (VERs) have been studied in rabbits and rats. VERs reflect postsynpatic removal activity. VER recordings consist of a series of positive and negative waves that are derived from the EEG by averaging signals that are time-locked to a photic stimulus. The recording of visual evoked potentials has been shown to be a reliable, quick, non-invasive method of quantifying neurologic changes during the evolution of hepatic encephalopathy (HE). Furthermore, the pattern of visual evoked potentials associated with HE is due to fulminant hepatic failure (FHF) is similar to that found in postictal coma and coma induced by barbituates, benzodiazepines (BZ) and the γ-aminobutyric acid (GABA) agonist, muscimol, but differs fundamentally from those associated with ether-induced coma or the encephalopathy induced by infusing a variety of potential neurotoxins in liver failure, either singly or in combination (ammonia, a mercaptan precurser, a short chain fatty acid).
As barbituates, BZ and GABA agonists are known to mediate their neuroinhibitory effects by augmenting chloride ion conductance across postsynaptic neural membranes as a consequence of their activating the GABA inhibitory neurotransmitter system, these observations suggest that the pattern of postsynaptic neuronal activity in HE due to FHF is similar to that associated with activation of GABA-ergic neural mechanisms. Furthermore, these observations may have clinical implications since the administration of either a GABA antagonist (bicuculline) or a BZ receptor antagonist (Ro 15-1788) has been shown to ameliorate HE due to FHF in rabbits, both clinically and electrophysiologically (improvement in the abnormal VER trace).

GABA metabolism and the status of the GABA neurotransmitter system have been investigated in relation to HE due to FHF. It has been shown that (i) a major source of GABA in peripheral blood is the enteric bacterial flora, (ii) plasma levels of GABA are increased several fold in HE; (iii) as the liver fails plasma levels of GABA become elevated before the onset of HE; and (iv) the density of receptors for GABA on postsynaptic neural membranes in the brain appears to be increased in acute liver failure. To determine whether GABA in plasma can gain access to the brain, the transport of the non-metabolized isomer of GABA, α-aminobutyric acid (AIB), across the blood brain barrier was measured by a computer-assisted quantitative autoradiographic technique. Blood-to-brain transport of ^{14}C-AIB is minimal in normal rabbits but with the onset of acute liver failure it increases markedly in specific gray matter areas of the brain prior to the onset of HE. Furthermore, although GABA is rapidly metabolized in vivo, it has been shown in rabbits, using modifications of the Oldendorf technique, which included a 4-second decapitation time and the use of a vascular marker, that the brain uptake index for GABA is increased two to threefold in acute liver failure. These findings suggest

that as liver fails plasma GABA derived from gut bacteria crosses abnormally permeable blood-brain barrier before the onset of HE and hence may contribute to the neural inhibition of HE. An increase in the number of GABA receptors in HE may be associated with enhanced sensitivity of the brain to GABA-ergic neural inhibition.

The number of binding sites for BZ on postsynaptic membranes also appears to be increased in HE, suggesting that the BZ binding site and GABA receptors may be regulated as a single operational unit. Increased sensitivity to BZ observed in liver failure may be due to to the presence of more BZ receptors permitting increased drug effect.

Clearance of ^{3}H-GABA from plasma is markedly decreased in acute liver failure. GABA binds specifically to the high affinity acceptor of the A amino acid transport system of isolated hepatocytes. Thus, hepatocellular injury may lead to impaired hepatic extraction of GABA which could contribute to increased plasma GABA. Increased plasma GABA levels are found in humans with acute or chronic hepatocellular failure and tend to be particularly high in cirrhotics following a gastrointestinal hemorrhage.

Studies of the binding of GABA to post-synaptic neural membranes prepared from brains obtained at human autopsies indicate that the affinities of GABA receptors are different in patients dying with chronic hepatocellular failure from those of patients dying from a variety of causes unrelated to the liver.

Hyperammonemic encephalopathy differs fundamentally from HE due to FHF in terms of the associated changes in visual evoked potentials as well as the densities of brain receptors not only for GABA but also for the excitatory neurotransmitter glutamate. In HE due to FHF there appears to be increases in the density of receptors for inhibitory amino acid neurotransmitters (not only GABA but also glycine), selective decreases in the density of receptors for excitatory amino acid neurotransmitters (glutamate, asparate and kainic acid) and no changes in the density of muscarinic acetylcholine, μ-opiate, δ-opiate or dopamine receptors. These findings are compatible with increased inhibitory neurotransmission and decreased excitatory neurotransmission contributing to the neural inhibition of HE due to FHF.

In rats the construction of a large end-to-side portacaval anastomosis (PCA) is followed by marked liver atrophy but no overt encephalopathy. Rats with a PCA who are gavaged with blood develop overt encephalopathy. Rats with a PCA who are gavaged with blood and have (CCl_4-induced) cirrhosis or a 50% hepatectomy develop more severe encephalopathy. The encephalopathy in these animals appears to be a model of portal systemic encephalopathy (PSE). The VER pattern in this model of PSE is different from that in rats with HE due to (thioactamide-induced) FHF raising the possibility that the neural mechanisms that mediate PSE may not be identified to those that mediate HE due to FHF.

In rats (urease-induced) gross hyperammonemia induces seizures. However in rats with a PCA (and liver atrophy) gross hyperammonemia induces a syndrome of neural inhibition (without seizures) in which the VER pattern resembles that in the model of PSE.

II. Studies of Cellular Immune Function in Primary Biliary Cirrhosis

The role of abnormal immune mechanisms in the pathogenesis and perpetuation of the hepatobiliary lesion of primary biliary cirrhosis (PBC) is being studied. Recent studies have been designed to determine whether there is a defect in immune regulation in patients with PBC. T lymphocyte-mediated help and suppression of pokeweed mitogen-induced immunoglobin (Ig) synthesis by cultured B lymphocytes have been quantitated in vitro using sensitive radioimmunoassays to measure IgG and IgM. The results suggest that there is deficient suppresion of Ig synthesis in PBC due to a suppressor T cell defect.

It has been shown that T cells can be induced to proliferate when cultured with either autologous or allogeneic irradiated B cells. These phenomena are known as the autologous and allogeneic mixed lymphocyte reactions (MLR), respectively. In patients with PBC, the mean autologous, but not the mean allogenic, MLR was found to be significantly less than the corresponding mean for controls. Thus, deficiencies of both suppressor T cell function and the autologous MLR are found in PBC. These deficiencies could be due to a common defect of cell interactions involved in the generation of suppressor effector cells. Such a defect could play an important role in the pathogenesis of PBC by providing an abnormal immunologic environment which predisposes to the development of autoimmune phenomena and a state of immune hyperresponsiveness.

The co-existence of IgA deficiency and PBC has been demonstrated. While it is possible that IgA deficiency may contribute to the development of PBC, the pathogenesis of PBC does not require IgA-dependent mechanisms.

Cytotoxicity mediated by circulating natural killer (NK) cells is reduced in PBC. This defect can be partially corrected by incubating PBC NK cells with interferon and appears to be due to a functional defect of cytolytic effector cells.

Complement receptor function in PBC has been assessed by quantitating the ability of peripheral blood monocytes to form rosettes with complement-coated sheep erythrocytes. PBC monocytes have a normal capacity to form rosettes but PBC serum in the presence or absence of normal serum inhibits rosette formation. This inhibition is probably mediated by an abnormally immunoreactive IgM present in PBC serum and does not depend on complement. An IgM that binds to receptors for C3b affords a potential explanation for the C3b-specific clearance defect by fixed macrophages in PBC.

Defects of humoral immunity attributable to activation of small subpopulations of B cells occur in PBC. For example, in PBC there is evidence compatible with an expanded clone of B cells that synthesizes mitochondrial antibodies with different antigenic specificities from those synthesized (under appropriate conditions) by normal B cells. [E.A. Jones, S.P. James (NIAID), M.I. Avigan (NIAID), J.H. Hoofnagle (NIDDK) and W. Strober (NIAID)].

III. Studies of Protease Inhibitor (Pi) Phenotypes

Pi phenotypes and serum α-1-antitrypsin (α1AT) concentrations have been determined in 80 unselected southern African Black patients with hepatocellular carcinoma and 103 age, sex and tribally matched control subjects. Non-MM phenotypes were present in 8.7% of patients with hepatocellular carcinoma and 12.6% of controls. The heterozygous PiZ carrier state was present in 5.0% of patients with hepatocellular carcinoma and 1.9% of controls; no subjects had the homozygous PiZZ phenotype. No patient with hepatocellular carcinoma had a subnormal serum 1AT concentration as assessed by rocket immunoelectrophoresis. The four patients with the heterozygous PiZ phenotype did not have fibrolamellar carcinomas. It is inferred that 1AT deficiency does not play an etiologic role in hepatocellular carcinoma in souther African Blacks. [E.A Jones, J. Vergalla and M.C. Kew (not NIH)].

IV. Controlled Trial of Chlorambucil Therapy in Primary Biliary Cirrhosis

Primary biliary cirrhosis (PBC) is a disease of unknown etiology characterized by slowly progressive intrahepatic cholestasis due to non-suppurative, presumably autoimmune, destruction of septal and the larger interlobular bile ducts. When the disease is symptomatic the intrahepatic cholestasis is almost invariably progressive and culminates in death due to hepatocellular failure. Because certain other autoimmune diseases appear to respond favorably to alkylating agents (but not to azathioprine), a controlled trial of chlorambucil therapy for patients with symptomatic PBC has been conducted. Twenty-four patients (23 women, 1 man; ages 34-63) were admitted to this trial: 13 were randomized to receive chlorambucil therapy (0.5-4.0 mg/day) and 11 to the control (no treatment) group. The dose of chlorambucil was adjusted to reduce the peripheral blood lymphocyte count by 50% and maintain the polymorphonuclear leukocyte count above 1000 per c.mm. All patients have been followed for 3-6 years (mean = 55 months). During follow-up, two patients have died: both in the control group. The mean serum bilirubin levels remained almost constant in the treated group but increased by an average of about 50% each year in the control group. Mean serum albumin values increased slightly in treated patients but decreased in control patients. Mean serum enzyme levels (aminotransferases and alkaline phosphatase) changed little in treated patients but tended to increase in controls. Mean serum immunoglobulin (IgM and IgG) levels decreased from elevated values to values within the normal range in all chlorambucil-treated patients, but did not change appreciably in control patients. Liver biopsy histopathology after one, two and four years revealed on average less inflammation, slightly less fibrosis and less progression of the stage of disease in the treated than in the control patients. Potential side effects of chlorambucil therapy included the onset of menopause in two patients, localized herpes simplex or zoster in 3 and, in 4 patients, persistent leukopenia or thrombocytopenia requiring discontinuation of the drug. These findings strongly suggest that chlorambucil therapy retards the progression of primary biliary cirrhosis, and they provide an impetus to search for safer

(e.g. noncarcinogeneic) and more effective immunosuppressive regimes for the treatment of this disease. A large-scale double-blind, placebo-controlled trial of chlorambucil is necessary to asess the effect of this drug on survival of patients with symptomatic PBC. [E.A. Jones, J.H. Hoofnagle, K.D. Mullen, R.N.M. MacSween (not NIH)].

V. Studies of the Natural History and Treatment of Chronic Type B Hepatitis

A cohort of patients with chronic type B hepatitis is being evaluated and followed to determine the long-term natural history of this common form of chronic liver disease. Selected patients have been entered into therapeutic trials in which antiviral or immunomodulatory agents have been administered. A randomized controlled trial of a four month course of alpha interferon in 45 patients with chronic type B hepatitis has recently been completed. In this trial, patients were randomized to receive (A) 5 million units of interferon sc daily for four months, (B) 10 million units of interferon sc every other day for four months, or (C) no therapy. All patients have been followed for one year and control patients have been "crossed over" to receive a four month course of interferon after evaluation following the intial year of follow up. During the period of therapy, 10 of the 31 treated (32%) but only one of 14 (7%) untreated patients lost serological markers of active hepatitis B viral replication (serum HBV-DNA and DNA polymerase activity). This difference was not statistically significant ($p = .09$). Among 10 control subjects crossed over to treatment, 4 (40%) responded with clearance of HBV-DNA from serum. Thus, the response rate to alpha interferon alone, given for a four month period, is low. Analysis of factors that might predict whether patients would respond to alpha interferon in the controlled trial as well as in our other studies of interferon therapy for this disease demonstrated that female sex and height of serum asparate aminotransferase activity (AST:SGOT) were the two best predictors of a favorable response. [J.H. Hoofnagle, K.D. Mullen, D.B. Jones, V. Rustgi, A. DiBisceglie, E.A. Jones].

VI. Studies of the Natural History and Treatment of Chronic Non-A, Non-B Hepatitis

Patients with well-documented chronic non-A, non-B hepatitis are being evaluated to determine the long-term natural history of this common form of chronic liver disease. A cohort of such patients is available to evaluate experimental therapies for this disease. To date nine patients with chronic non-A, non-B hepatitis have been treated with recombinant human alpha interferon for periods ranging from 2 months to one year. Seven of the nine patients have shown a dramatic decrease in serum aminotransferase levels during therapy, their levels falling from values 3 to 10 times the upper limit of the normal range to normal (6 patients) or near normal (1 patient). Follow up liver biopsies have been obtained from two patients, both of which demonstrate marked improvement in the hepatitis disease activity (a decrease in both inflammation and hepatocellular necrosis). The dose and schedule of administration of interferon are currently being adjusted to identify the optimal regimen to achieve maximal benefit (as monitored by serum aminotransferase levels) with minimal side effects, and

discomfort. The optimum dose appears to be 2 million units (mu) given three times per week. A prospective, randomized, placebo-controlled trial of a six month course of human alpha interferon in patients with chronic non-A, non-B hepatitis is planned. [J.H. Hoofnagle, K.D. Mullen, D.B. Jones, V. Rustgi, A. DiBisceglie, E.A. Jones].

VII. Immunologic Studies in Chronic Viral Hepatitis

Immunological factors seem to be important in determining the course and outcome of both acute and chronic viral hepatitis. Furthermore promising therapies for chronic viral hepatitis have profound effects on immune function and sustained responses to therapy may depend largely on restoration of normal immune responsiveness. The role of immunologic mechanisms in determining the course and ultimate outcome of viral hepatitis is being studied and the effects of therapies on the immune system are being evaluated. Serial studies of cellular immune function were performed on patients with acute viral hepatitis and compared to analogous results obtained in patients with chronic type B hepatitis. In addition, the immunological status of patients with chronic type B hepatitis has been assessed and the effect of immunosuppressive as well as antiviral therapy on immunological function in these patients has been studies prospectively. [J.H. Hoofnagle, E.A. Jones, K.D. Mullen, D.B. Jones, V. Rustgi, A. DiBisceglie].

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 53001-16 DDB
formerly
Z01 AM 53001-15 DDB

PERIOD COVERED: October 1, 1985 to Sept. 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies of Membrane Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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Others:	R. T. Jensen	Senior Investigator	DDB, NIDDK
	P. N. Maton	Visiting Scientist	DDB, NIDDK
	S. A. Wank, J. I. Slaff	Medical Staff Fellows	DDB, NIDDK
	R. Vinayek, H. Frucht	Medical Staff Fellows	DDB, NIDDK
	Z-C. Zhou, M. Younes, D-H. Yu	Visiting Fellows	DDB, NIDDK
	T. Garvey, D. Kasbekar, D. Menozzi	Guest Workers	DDB, NIDDK
	P. Heinz-Erian, T. von Schrenck	Guest Workers	DDB, NIDDK
	S. W. Jones, V. E. Sutliff	Chemists	DDB, NIDDK

COOPERATING UNITS *(if any)*

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Digestive Diseases Branch

SECTION
Section on Gastroenterology

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	3.6	1.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The broad categories which are included in the project are: 1) Characterizing functionally the mechanism by which various substrates cross the plasma membrane of different mammalian cells; 2) identifying the metabolic and humoral factors which influence the transport of various substrates across the plasma membrane; 3) developing techniques which will distinguish between binding of a substrate to the membrane and translocation of the substrate across the membrane; 4) characterizing the mechanism by which the membrane transport of various substrates is altered in certain diseases; and 5) relating these alterations of membrane transport to the pathogenesis and clinical manifestations of the disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 53002-14 DDB
formerly
Z01 AM 53002-13 DDB

PERIOD COVERED
October 1, 1985 to Sept. 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Gastrointestinal Hormones

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. D. Gardner	Chief	DDB, NIDDK
Others:	R. T. Jensen	Senior Investigator	DDB, NIDDK
	P. N. Maton	Visiting Scientist	DDB, NIDDK
	S. A. Wank, H. Frucht	Medical Staff Fellows	DDB, NIDDK
	R. Vinayek, J. I. Slaff	Medical Staff Fellows	DDB, NIDDK
	Z-C. Zhou, D-H. Yu	Visiting Fellows	DDB, NIDDK
	D. Kasbekar. M. Younes, D. Menozzi	Guest Workers	DDB, NIDDK
	P. Heinz-Erian, T. von Schrenck	Guest Workers	DDB, NIDDK
	S. Jones, V. Sutliff	Chemists	DDB, NIDDK

COOPERATING UNITS *(if any)*
Dept. of Chemistry, Case-Western Reserve Univ., Cleveland, Ohio
Div. of Cellular Biology, Kennedy Institute for Rheumatology, London, England

LAB/BRANCH
Digestive Diseases Branch

SECTION
Section on Gastroenterology

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.6	4.0	1.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In vitro systems are being used to study the mechanism of action of gastrin, secretin, cholecystokinin, bombesin, substance P and vasoactive intestinal peptide with their specific membrane receptors.

Clinical investigators are directed toward developing alternative forms of therapy for and elucidating the pathogenesis of disorders characterized by ectopic production of gastrointestinal hormones (e.g., Zollinger-Ellison syndrome and pancreatic cholera).

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 53004-14 DDB
formerly
Z01 AM 53004-13 DDB

PERIOD COVERED
October 1, 1985 to Sept. 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cyclic Nucleotide Mediated Functions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Jerry D. Gardner	Chief	DDB, NIDDK
Others:	R. T. Jensen	Senior Investigator	DDB, NIDDK
	P. N. Maton	Visiting Scientist	DDB, NIDDK
	S. Wank, J. Slaff, R. Vinayek	Medical Staff Fellows	DDB, NIDDK
	Z-C. Zhou, D-H. Yu	Visiting Fellows	DDB, NIDDK
	D. Kasbekar, M. Younes. D. Menozzi	Guest Workers	DDB, NIDDK
	T. Garvey, T. von Schrenck, P. Heinz-Erian	Guest Workers	DDB, NIDDK
	S. Jones, V. Sutliff	Chemists	DDB, NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Digestive Diseases Branch

SECTION
Gastroenterology

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.6	5.0	1.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In vitro systems are being used to characterize the mechanism by which cyclic nucleotides alter cell function and to explore the mechanism of action of agents whose effect on cell function is mediated by cellular accumulation of cyclic nucleotides.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 53,501-13 DDB
formerly
Z01 AM 53,501-12 DDB

PERIOD COVERED
October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies Relating to the Pathogenesis of Hepatic Encephalopathy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

E. Anthony Jones, M.D.	Chief, Liver Diseases Section	DK:DDB
Others:		
Dr. J. Vergalla	Chemist	DK:DDB
Dr. K. D. Mullen	Medical Staff Fellow	DK:DDB
Dr. D. B. Jones	Medical Staff Fellow	DK:DDB
Dr. M. Rossle	Guest Researcher	DK:DDB
Dr. S. Gammal	Guest Researcher	DK:DDB

COOPERATING UNITS *(if any)*
RR:NICHHD (Dr. P. J. Munson); LBC:NIDDK (Dr. P. Skolnick); DCBD:NCI (Dr. D. Covell); LPM:NINCDS (Dr. J. Barker)

LAB/BRANCH
Digestive Diseases Branch

SECTION
Liver Diseases Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The abnormal pattern of visual evoked responses (VERs) in rabbits with hepatic encephalopathy (HE) due to fulminant hepatic failure (FHF) resembles that associated with coma induced by a barbiturate, a benzodiazepine or a γ-amino-butyric acid (GABA) agonist. As these drugs induce neural inhibition by interacting with binding sites on the GABA receptor complex on postsynaptic neural membranes, these findings suggest that the pattern of neuronal activity in HE may resemble that associated with activation of the GABA inhibitory neurotransmitter system. Outside the CNS the main source of GABA is gut bacteria and the main site of its catabolism is the liver. When FHF was induced in rabbits the onset of HE was preceded by an increase in the plasma levels of GABA and by a nonspecific increase in the permeability of the blood brain barrier (BBB) to a nonmetabolized isomer of GABA. To take account of the rapid metabolism of GABA a modified Oldendorf technique, which employed the use of a vascular marker, has been used to demonstrate that the brain uptake index for GABA itself is increased in HE. FHF was associated with significant increases in the densities of brain receptors for the inhibitory amino acid neurotransmitters, and for benzodiazepines (BZ), with significant decreases in the densities of brain receptors for the excitatory amino acid neurotransmitters, and with changes in the composition of neural membranes. Both a GABA receptor antagonist and a BZ receptor antagonist induced an amelioration of HE due to FHF both clinically and electrophysiologically (VER pattern). These findings suggest that in acute liver failure: (i) plasma GABA gains access to the brain through a permeable BBB; (ii) the brain may be less sensitive to excitatory amino acid neurotransmitters and more sensitive to inhibitory amino acid neurotransmitters and to BZ and (iii) an endogenous BZ ligand may contribute to the neural inhibition of HE by augmenting GABAergic tone.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 53,503-12 DDB
formerly
Z01 AM 53,503-11 DDB

PERIOD COVERED
October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Immunologic Studies of Primary Biliary Cirrhosis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

E. Anthony Jones, M.D.	Chief, Liver Diseases Section	DK:DDB
Others:		
Jay H. Hoofnagle	Senior Investigator	DK:DDB
John Vergalla	Chemist	DK:DDB

COOPERATING UNITS *(if any)*

LCI:NIAID (Dr. S. P. James and Dr. W. Strober)

LAB/BRANCH
Digestive Diseases Branch

SECTION
Liver Diseases Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.75	0.75	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Abnormal immune mechanisms are being studied in patients with primary biliary cirrhosis (PBC). T cell-mediated help and suppression of pokeweed mitogen-induced immunoglobulin synthesis by B cells have been studied using radio-immunoassays to measure IgG and IgM synthesized by cultures containing appropriate mixtures of different lymphocyte subpopulations in vitro. The ability of T cells to proliferate when cultured with either autologous or allogeneic irradiated B cells (mixed lymphocyte reactions) has been assessed. Results of these studies include the demonstration in PBC of (i) a diminished capacity of T cells to inhibit immunoglobulin synthesis in vitro and (ii) a deficiency of the autologous but not the allogeneic mixed lymphocyte reaction. These findings suggest that in PBC there is a fundamental defect in the interaction between autoreactive T cells and surface antigens on autologous non-T cells which leads to diminished activation of suppressor T cells and hence predisposes to a state of immune hyperresponsiveness. The coexistence of IgA deficiency and PBC has been documented. It is possible that IgA deficiency may contribute to the development of PBC, but the pathogenesis of PBC does not require IgA-dependent mechanisms. Sera from patients with PBC have been shown to contain a factor, probably an abnormally immunoreactive IgM, which blocks the binding of C3b-opsonized erythrocytes by monocytes. This finding affords a potential explanation for the C3b-receptor specific clearance defect by fixed macrophages in PBC. Patients with PBC have been shown to have diminished natural killer cell activity due to a functional defect of cytolytic effector cells. Defects of humoral immunity due to activation of sub-populations of B cells occur in this disease. For example, in PBC there is evidence compatible with the existence of an expanded clone of B cells that synthesize mitochondrial antibodies with different antigenic specificities from those synthesized by normal B cells. A disease-specific immunologic defect has yet to be defined in PBC.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 53,505-11 DDB
formerly
Z01 AM 53,505-10 DDB

PERIOD COVERED
October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies of Alpha-1-Antitrypsin Phenotypes and Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

E. Anthony Jones, M.D.	Chief, Liver Diseases Section	DK:DDB
Others:		
John Vergalla	Chemist, LDS	DK:DDB

COOPERATING UNITS *(if any)*

University of the Witwatersrand, Johannesburg, South Africa
(Dr. M. C. Kew)

LAB/BRANCH
Digestive Diseases Branch

SECTION
Liver Diseases Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Protease inhibitor (Pi) phenotypes have been determined using isoelectric focusing on polyacrylamide gel in populations of normal subjects and patients with rheumatoid arthritis, systemic lupus erythematosus, Sjogren's syndrome and hepatocellular carcinoma. Of 80 unselected southern African Black patients with hepatocellular carcinoma, the incidence of aberrant (non-MM) phenotypes was 8.7%. In 103 age-, sex- and tribally-matched control subjects the corresponding incidence was 12.6%. None of the patients or controls had the PiZZ phenotype. 5% of patients and 1.9% of controls were heterozygous carriers of the Z gene. No patient with hepatocellular carcinoma had a sub-normal serum concentration of alpha-1-antitrypsin, as assessed by rocket immuno-electrophoresis. The four patients with the heterozygous Z phenotype did not have fibrolamellar carcinomas. These findings suggest that alpha-1-antitrypsin deficiency does not play an etiologic role in hepatocellular carcinoma in southern African Blacks.

THIS PROJECT IS CURRENTLY INACTIVE

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 53,508-09 DDB
formerly
Z01 AM 53,508-08 DDB

PERIOD COVERED: October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Studies of Hepatic Receptors for Glycoproteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

E. Anthony Jones, M.D.	Chief, Liver Diseases Section	DK:DDB
Others:		
John Vergalla, B.A.	Chemist, LDS	DK:DDB

COOPERATING UNITS *(if any)*

LAB/BRANCH: Digestive Diseases Branch

SECTION: Liver Diseases Section

INSTITUTE AND LOCATION: NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The cellular location and carbohydrate specificities of a glycoprotein recognition system on rat hepatic sinusoidal cells have been determined. Purified preparations of endothelial, Kupffer and parenchymal cells have been prepared by in situ collagenase liver perfusion, centrifugation on Percoll gradients and centrifugal elutriation. ^{125}I-labeled agalactoorosomucoid (AGOR), an N-acetylglucosamine-terminated glycoprotein, was selectively and specifically taken up in vitro by endothelial cells. Glucose and a glucose-albumin conjugate competitively inhibited this uptake process over a wide range of concentrations. Uptake by cells from fasted rats was enhanced, but uptake by cells from fasted or fed diabetic rats was normal. The in vivo hepatic uptake and catabolism of ^{125}I-AGOR were slower in diabetic than normal rats. It is inferred that 1) the hepatic receptors which recognize N-acetylglucosamine/mannose terminated glycoproteins are located predominantly on endothelial cells, 2) these receptors are glucose sensitive, 3) fasting increases the number of these receptors and 4) diabetes mellitus abolishes this effect of fasting and impairs the function of this receptor in vivo. These findings suggest a mechanism for abnormal glycoprotein metabolism in diabetes mellitus. This carbohydrate recognition system may play an important role in the removal of potentially autodestructive glycoprotein lysosomal hydrolases and other glycoprotein enzymes from the circulation under normal physiological conditions and in disease states.

THIS PROJECT IS CURRENTLY INACTIVE

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 53,509-08 DDB
formerly
Z01 AM 53,509-07 DDB

PERIOD COVERED
October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Studies of the Natural History and Treatment of Chronic Type B Hepatitis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Jay H. Hoofnagle, M.D.	Senior Investigator, Liver Diseases Section	DK:DDB
Others:		
E. Anthony Jones, M.D.	Chief, LDS	DK:DDB
Kevin D. Mullen, M.D.	Medical Staff Fellow, LDS	DK:DDB
D. Brian Jones, M.D.	Medical Staff Fellow, LDS	DK:DDB
Vinod Rustgi, M.D.	Medical Staff Fellow, LDS	DK:DDB
Adrian Di Bisceglie, M.D.	Medical Staff Fellow, LDS	DK:DDB

COOPERATING UNITS (if any)
Georgetown University, Washington, D.C. (Dr. John Gerin)
LID:NIAID (Dr. Stephen Feinstone)
Walter Reed Army Institute of Research, Washington, D.C. (Dr. Maria Sjogren)

LAB/BRANCH
Digestive Diseases Branch

SECTION
Liver Diseases Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3	2	1

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A cohort of patients with chronic type B hepatitis is being evaluated and followed to determine the long-term natural history of this common form of chronic liver disease. Selected patients have been entered into the therapeutic trials in which antiviral or immunomodulatory agents have been administered. A randomized controlled trial of a four month course of alpha interferon in 45 patients with chronic type B hepatitis has recently been completed. In this trial, patients were randomized to receive either (A) 5 million units sc of interferon sc daily for four months, (B) 10 million units sc of interferon every other day for four months, or (C) no therapy. All patients have been followed for one year and control patients have been "crossed over" to receive a four month course of interferon after evaluation following the initial year of follow up. During the period of therapy, 10 of the 31 treated (32%) but only one of 14 (7%) untreated patients lost serological markers of active hepatitis B viral replication (serum HBV-DNA and DNA polymerase activity). This difference was not statistically significant ($p = .09$). Among 10 control subjects crossed over to treatment, 4 (40%) responded with clearance of HBV-DNA from serum. Thus, the response rate to alpha interferon alone, given for a four month period, is low. Analysis of factors that might predict whether patients would respond to alpha interferon in the controlled trial as well as in our other studies of interferon therapy for this disease demonstrated that female sex and height of serum aspartate aminotransferase activity (AST:SGOT) were the two best predictors of a favorable response.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 53,510-07 DDB
formerly
Z01 AM 53,510-06 DDB

PERIOD COVERED
October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies of the Natural History and Treatment of Chronic Non-A, Non-B Hepatitis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Jay H. Hoofnagle, M.D.	Senior Investigator, Liver Diseases Section	DK:DDB
Others:		
E. Anthony Jones, M.D.	Chief, LDS	DK:DDB
Kevin D. Mullen, M.D.	Medical Staff Fellow, LDS	DK:DDB
D. Brian Jones, M.D.	Medical Staff Fellow, LDS	DK:DDB
Vinod Rustgi, M.D.	Medical Staff Fellow, LDS	DK:DDB
Adrian Di Bisceglie, M.D.	Medical Staff Fellow, LDS	DK:DDB

COOPERATING UNITS *(if any)*
NIH Blood Bank (Dr. Harvey J. Alter)
LCI:NIAID (Dr. Stephen E. Straus)
Armed Forces Institute of Pathology, Washington, D.C. (Dr. Kamal Ishak)

LAB/BRANCH
Digestive Diseases Branch

SECTION
Liver Diseases Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.75	0.75	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Patients with well-documented chronic non-A, non-B hepatitis are being evaluated to determine the long-term natural history of this common form of chronic liver disease. A cohort of such patients is available to evaluate experimental therapies for this disease. To date nine patients with chronic non-A, non-B hepatitis have been treated with recombinant human alpha interferon for periods ranging from 2 months to one year. Seven of the nine patients have shown a dramatic decrease in serum aminotransferase levels during therapy, their levels falling from values 3 to 10 times the upper limit of the normal range to normal (6 patients) or near-normal (1 patient). Follow up liver biopsies have been obtained from two patients, both of which demonstrate marked improvement in the hepatitis disease activity (a decrease in both inflammation and hepatocellular necrosis). The dose and schedule of administration of interferon are currently being adjusted to identify the optimal regime to achieve maximal benefit (as monitored by serum aminotransferase levels) with minimal side effects and discomfort. The optimum dose appears to be 2 million units (mu) given three times per week. A prospective, randomized, placebo-controlled trial of a six month course of human alpha interferon in patients with chronic non-A, non-B hepatitis is planned.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 53,511-07 DDB
formerly
Z01 AM 53,511-06 DDB

PERIOD COVERED: October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Controlled Trial of Chlorambucil in Primary Biliary Cirrhosis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

E. Anthony Jones, M.D.	Chief, Liver Diseases Section	DK:DDB
Others:		
Jay H. Hoofnagle, M.D.	Senior Investigator, LDS	DK:DDB
Kevin D. Mullen, M.D.	Medical Staff Fellow	DK:DDB
Vinod Rustgi, M.D.	Medical Staff Fellow	DK:DDB
David B. Jones, M.D.	Medical Staff Fellow	DK:DDB

COOPERATING UNITS (if any)

University Department of Pathology, Western Infirmary, Glasgow, U.K.
(Dr. R. N. M. MacSween)

LAB/BRANCH: Digestive Diseases Branch

SECTION: Liver Diseases Section

INSTITUTE AND LOCATION: NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Primary biliary cirrhosis (PBC) is a disease of unknown etiology characterized by slowly progressive intrahepatic cholestasis due to non-suppurative, presumably autoimmune, destruction of septal and the larger interlobular bile ducts. Because certain other autoimmune diseases appear to respond favorably to alkylating agents, a controlled trial of chlorambucil therapy for patients with symptomatic PBC has been conducted. Twenty-four patients were admitted to this trial: 13 were randomized to receive chlorambucil therapy (0.5-4.0 mg/day) and 11 to the control (no treatment) group. The dose of chlorambucil was adjusted to reduce the peripheral blood lymphocyte count by 50% and maintain the polymorphonuclear leukocyte count above 1000 per c.mm. All patients have been followed for 3-6 years (mean = 55 months). During follow-up, two patients died: both were controls. Mean serum bilirubin levels remained almost constant in the treated group but increased by an average of about 50% each year in the controls. Mean serum albumin values increased slightly in treated patients but decreased in controls. Mean serum transaminase and alkaline phosphatase levels changed little in treated patients, but tended to rise in controls. Mean serum immunoglobulin (IgM and IgG) levels decreased from elevated values to values within the normal range in all chlorambucil-treated patients, but did not change appreciably in controls. Liver biopsy histopathology after one, two and four years revealed less inflammation, slightly less fibrosis and less progression of the stage of disease in the treated than in the control patients. Potential side effects of chlorambucil therapy included the onset of menopause in two patients, localized herpes simplex or zoster in 3 and, in 4 patients, persistent leukopenia or thrombocytopenia requiring discontinuation of the drug. These findings strongly suggest that chlorambucil therapy retards the progression of primary biliary cirrhosis, and they provide an impetus to search for safer (e.g. noncarcinogenic) and more effective immunosuppressive regimes for the treatment of this disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 53,514-03 DDB
formerly
Z01 AM 53,514-02 DDB

PERIOD COVERED
October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Immunological Studies in Chronic Viral Hepatitis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Jay H. Hoofnagle, M.D.	Senior Investigator, Liver Diseases Section	DK:DDB
Others:		
E. Anthony Jones, M.D.	Chief, LDS	DK:DDB
Kevin D. Mullen, M.D.	Medical Staff Fellow, LDS	DK:DDB
D. Brian Jones, M.D.	Medical Staff Fellow, LDS	DK:DDB
Vinod Rustgi, M.D.	Medical Staff Fellow, LDS	DK:DDB
Adrian Di Bisceglie, M.D.	Medical Staff Fellow, LDS	DK:DDB

COOPERATING UNITS *(if any)*

LAB/BRANCH
Digestive Diseases Branch

SECTION
Liver Diseases Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1.5	.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Immunological factors seem to be important in determining the course and outcome of both acute and chronic viral hepatitis. Furthermore promising therapies for chronic viral hepatitis have profound effects on immune function and sustained responses to therapy may depend largely on restoration of normal immune responsiveness. The role of immunologic mechanisms in determining the course and ultimate outcome of viral hepatitis is being studied and the effects of therapies on the immune system are being evaluated. Serial studies of cellular immune function were performed on patients with acute viral hepatitis and compared to analogous results obtained in patients with chronic type B hepatitis. In addition, the immunological status of patients with chronic type B hepatitis has been assessed and the effect of immunosuppressive as well as antiviral therapy on immunological function in these patients has been studied prospectively.

GPO 904-917

ANNUAL REPORT OF THE

MOLECULAR, CELLULAR, AND NUTRITIONAL ENDOCRINOLOGY BRANCH

NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

The MCNEB continues basic and clinical investigations in the areas of molecular regulation and neuroendocrinology (Molecular Regulation and Neuroendocrinology Section, Bruce D. Weintraub, Chief); experimental diabetes, metabolism and nutrition (Experimental Diabetes, Metabolism and Nutrition Section, Samuel W. Cushman, Chief); and growth and development (Growth and Development Section, Matthew M. Rechler, Chief). The Branch has had many visiting fellows and associates, as well as international collaborations with the University of Milan, Italy; University of Marseilles, France; University of Lisbon, Portugal; Karolinska Institute, Sweden; Institute of Organic Chemistry, Padova, Italy; Nankai University, Tianjin, Peoples Republic of China; Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague, CSSR; Postgraduate School of Obstetrics and Gynaecology, University of Auckland, New Zealand; University of Naples, Italy; Department of Medicine, University of Gothenburg, Sweden; Endocrine Institute, Rambam Medical Center, Haifa, Israel; Department of Biochemistry, The University of Newcastle upon Tyne, England.

Dr. Bruce D. Weintraub was elected into the Association of American Physicians.

I. GLYCOPROTEIN HORMONES: SYNTHESIS, PROCESSING, REGULATION AND ACTION

A. Mechanism and Inhibition of Thyrotropin Subunit Glycosylation and Carbohydrate Processing.

We continue to use deoxynojirimycin (dN), an inhibitor of glycoprotein processing to study the role of carbohydrate maturation in (1) the secretion and degradation of TSH and its free α subunit and (2) the sorting of TSH and free α along specific pathways through the cell. Hypothyroid mouse pituitary halves were incubated with [^{35}]methionine then incubated in isotope-free media for various chase periods up to 18 hours. The media and lysates were sequentially immunoprecipitated with anti-growth hormone and prolactin, anti-TSHβ, anti-LHβ, and anti-LHα, followed by polyacrylamide gel electrophoresis. In the cell TSH becomes resistant to endocglycosidase H faster than free α with or without dN, indicating more rapid carbohydrate processing. In contrast, free α disappears from the cell and appears in the media faster than 5ΔH with or without dN. However, dN selectively inhibited the secretion and enhanced the intracellular degradation of TSH while having little effect on the secretion of free α, growth hormone or prolactin. These data suggest separate secretory routes for TSH and free α. They also suggest that a specific carbohydrate configuration may be required for TSH secretion and for its protection from intracellular proteolysis.

. . . . B. S. Stannard, N. Gesundheit, B. D. Weintraub

B. Analysis of TSH Carbohydrate Structure: Animal Models

Serial lectin affinity chromatography was used analytically to characterize the carbohydrate moiety on TSH molecules secreted by hypothyroid mouse pituitaries. TSH glycopeptides were first chromatographed on concanavalin A (conA)-Sepharose; glycopeptides that failed to bind were then chromatographed on erythro-phytohemagglutinin (E-PHA), which will bind oligosaccharides containing a bisecting N-acetylglucosamine residue; glycopeptides that failed to bind to E-PHA were then chromatographed on pea lectin, which will bind certain fucosylated multiantennary oligosaccharides; finally, both glycopeptides that bound and failed to bind to pea lectin were subsequently chromatographed on leuko-phytohemagglutinin (L-PHA), which will bind certain multiantennary oligosaccharides containing outermost galactose residues. In addition, glycopeptides that bound weakly to conA, corresponding to complex biantennary oligosaccharides, were further chromatographed on pea lectin to determine the extent of their fucosylation. The lectin affinity profiles thus generated have suggested greater heterogeneity of TSH structure than has been previously appreciated, with 30-40% of secreted [^{3}H]mannose-labeled TSH glycopeptides containing multiple antennas. The biochemical mechanisms for the synthesis and the functional significance of these multiantennary forms will be explored in future investigations.

The effect of physiological concentrations of TRH on TSH carbohydrate structure was explored. Lectin affinity analysis for TSH glycopeptides secreted both with and without *in vitro* TRH (10^{-7} M) were contrasted. These profiles revealed that there were no apparent differences in the content of bisecting N-acetylglucosamine residues or in the degree of core fucosylation. The striking difference was that TSH glycopeptides secreted in the presence of TRH demonstrated greater ConA binding, suggesting the presence of more biantennary complex or truncated hybrid oligosaccharides. These data suggest that TRH may accelerate secretion of TSH and not permit processing of TSH carbohydrate to more complex, multiantennary forms or that TRH specifically inhibits the action of one or more N-acetylglucosaminyltransferase(s) and thus enzymatically limits the degree of TSH carbohydrate branching.

. . . . N. Gesundheit, B. D. Weintraub

C. Analysis of TSH Carbohydrate Structure: Human Models

To provide clinical correlation with human TSH biosynthesis, we studied three patients with TSH-secreting pituitary adenomas and analyzed TSH secreted *in vitro* by tumor tissue that had been removed at surgery. Where possible, the methods of serial lectin affinity chromatography were utilized to provide more detailed analysis of the carbohydrate structure of TSH secreted by these human tumors. Consistent with the hypothyroid mouse model, a large percentage (52-70%) of tumor-derived TSH glycopeptides failed to bind to con A. In one tumor where these could be characterized, approximately half of the glycopeptides that failed to bind to con A were bound to pea lectin, suggesting the presence of certain fucosylated multiantennary forms. One patient demonstrated a good response to TRH *in vivo* and her tumor was incubated both with and without 10^{-7} M TRH *in vitro*. TRH increased the percentage of secreted TSH glycopeptides that bound to conA (from 30% to 68%) due to

greater numbers of both biantennary as well as high-mannose and/or hybrid forms; this change was confirmed with a variety of tritiated sugar precursors (glucosamine, mannose and fucose). As in the animal model, this suggests that TRH may accelerate TSH secretion and not permit more complex carbohydrate branching by either enzymatic or non-enzymatic mechanisms. Future efforts may explore if decreased conA binding, seen in tumor-derived TSH glycopeptides, provides a useful clinical tool to assist in the diagnosis of TSH-secreting tumors.

. . . . N. Gesundheit, B. D. Weintraub

D. Hypothalamic Regulation of TSH Secretion, Synthesis and Carbohydrate Structure

The effects of hypothalamic deafferentation on TSH synthesis were studied by making cuts of 180° arc in the anterior hypothalamus (N=18) or sham cuts (N=12) in rats. After 21 days, pituitaries were incubated with [^{35}S] methionine, [^{3}H]glucosamine, with or without 10^{-8} M TRH for 24 h. TSH and free α subunits were immunoprecipitated and analyzed by gel electrophoresis. The results of these studies indicated: 1) anterior hypothalamic deafferentation decreased basal TSH protein and carbohydrate synthesis; 2) such deafferentation increased sensitivity to TRH stimulation of TSH synthesis, most notably apoprotein synthesis; 3) TRH increased relative glycosylation of secreted TSH in both deafferented and sham groups. These data suggest that TRH plays a significant role in regulating basal TSH protein and carbohydrate synthesis, glycosylation of TSH subunits and subsequent bioactivity.

The effects of hypothalamic hypothyroidism created by paraventricular nuclei lesions and primary hypothyroidism on TSH carbohydrate structure were studied in rats. Bilateral lesions in the paraventricular nuclei (N=9) and sham lesions (N=7) were made and compared to thyroidectomized (N=6) and normal rats (N=6). After 2 weeks, pituitaries were incubated with ^{3}[H]glucosamine for 24 hr, then TSH was radioimmunoassayed and immunoprecipitated, pronase digested, desalted then analyzed by concanavalin A chromatography.

The results of these studies indicated: 1) Paraventricular nuclei lesions decreased secreted TSH and intrapituitary TSH. 2) Secreted and intrapituitary labeled TSH glycopeptides from the hypothalamic hypothyroidism group demonstrated increased weakly bound forms as compared to the sham group. 3) Secreted TSH glycopeptides from the primary hypothyroid group demonstrated increased unbound forms as compared to the normal group; there were no differences in intrapituitary forms. 4) TSH carbohydrate structure in the hypothalamic hypothyroid group markedly differed from the primary hypothyroid group: the former had more biantennary forms and the latter had a larger proportion of multiantennary or bisecting forms.

. . . . T. Taylor, B. D. Weintraub

E. De novo protein and carbohydrate biosynthesis of TSH during postnatal ontogenesis.

Progressive increases per pituitary in the biosynthesis and secretion of the apoprotein and carbohydrate moieties of TSH were noted during postnatal ontogenesis. When normalized to pituitary mass, these data showed variable although not significant differences. In addition, free α/TSH increased significantly at 14 days in the apoprotein and at 25 days in the carbohydrate moieties before declining to adult values. Finally, the percent secretion of free α was greater than that of TSH at all ages of development. In summary: (1) The increase in TSH subunits during postnatal ontogenesis is primarily related to increasing pituitary mass. (2) Changes in free α/TSH may reflect dynamic alterations in free α subunit or other glycoprotein hormones during ontogeny. (3) Secretion of free α is more efficient than that of TSH at all ages of postnatal development.

. . . . P. W. Gyves, N. Gesundheit, B. D. Weintraub

F. Concanavalin A (con A) analysis of in vitro rat TSH carbohydrate structure and its regulation by TRH during development.

Newly secreted TSH was greater per pituitary in 56-day-old compared to 5-day-old rats. However, the younger animal had a greater proportion of intrapituitary TSH, manifested by a lower percent secretion of TSH. In addition, for both intrapituitary and secreted forms, the percentage of TSH glycopeptides not bound to con A was greater in 56-day-old compared to 5-day-old animals. A corresponding decrease in the older animals was noted in the percentage of TSH glycopeptides that bound to con A and eluted with α methylglucoside for both intrapituitary and secreted forms, while there was no change between the 2 ages in those forms that bound to con A and eluted with α methylmannoside. In the presence of 10^{-7} M TRH, the 5-day-old animal demonstrated a 2.2-fold increase/pituitary above controls in the amount of newly synthesized and secreted TSH, while the 56-day-old animal had a 2.4-fold increase. In addition, the 5-day-old animal demonstrated a proportional increase in all classes of secreted TSH glycopeptides, while the 56-day-old animal showed a qualitative alteration in its con A profile with a decrease in the percentage of TSH glycopeptides not bound to con A compared to controls. No change occurred in those forms that bound to con A and eluted with α methylmannoside. In summary: (1) Neonatal rats had a lower percent secretion of TSH compared to the adult, indicating an inefficient secretory process in the younger animal. (2) For both intrapituitary and secreted TSH, the adult contained a greater proportion of multiantennary and/or bisecting carbohydrate structures compared to the neonate. (3) In vitro TRH resulted in different secretory responses at these 2 ages. The neonatal rat demonstrated a proportional increase in all classes of TSH glycopeptides, while the adult showed a selective increase in the proportion of biantennary and/or truncated hybrid forms. These data suggest there are differences in TSH carbohydrate structure during postnatal ontogenesis, as well as different secretory responses to

regulatory influences. Correlation of these structural changes with bioactivity is currently under investigation.

. . . . P. W. Gyves, N. Gesundheit, B. D. Weintraub

G. Effect of various secretagogues on TSH carbohydrate structure.

A series of experiments was designed to examine the specificity and mechanisms of the TRH effect on TSH carbohydrate structure. Since previous studies of the mechanism of action of TRH were performed in GH3 cells and thyrotropic tumors, we employed a thyrotropic tumor model. Enzymatically dispersed thyrotropic tumor cells were metabolically labeled with [^{3}H]mannose and incubated for 24 hours in the absence or presence of one of the following secretagogues: 1) TRH, 2) Phorbol ester which stimulates secretion by acivation of protein kinase C, 3) KCl which causes secretion by membrane depolarization. TSH was analyzed by RIA and TSH glycopeptides were purified by immunoprecipitation and analyzed by con A affinity chromatography. TRH caused a 2-fold increase in TSH secretion and a significant change in the con A binding pattern, increasing the activity of labeled glycopeptides which bound tightly to con A. KCl caused a 3-fold increase in TSH secretion over control but did not change the con A binding profile. Phorbol ester stimulated a 2-fold increase in TSH secretion with a con A binding profile similar to, but not identical to TRH. Thus the TRH stimulated changes in the oligosaccharide structure of secreted TSH are specific and are not mediated by potassium and only in part by stimulation of protein kinase C.

. . . . J. B. Butler, N. Gesundheit, B. D. Weintraub

H. Differences in serum TSH bioactivity among normal individuals, patients with primary hypothyroidism and patients with TSH-secreting pituitary tumors.

Heterogeneity of the intrapituitary TSH molecules has since long been observed and lately both increased and decreased bioactivity of circulating human TSH has been described in specific pathological conditions. We have developed a new bioassay for TSH in human serum to evaluate TSH bioactivity in 4 normal individuals, 8 mildly to severely primary hypothyroid patients and in 5 patients with TSH-secreting pituitary tumors. Unpurified TSH in serum from hypothyroid and tumor patients showed no significant stimulation of cyclic AMP in cultured FRTL-5 rat thyroid cells. However, after immunopurification, TSH from the same patients showed potent stimulatory activity. Moreover, the immunoaffinity purification permitted up to 400-fold concentration of serum TSH, allowing bioactivity measurements even in certain normal sera. Among euthyroid subjects the bioactivity to immunoactivity (B/I) ratios varied from <0.25 to 0.30 and among primary hypothyroid patients from 0.34 to 1.21. An inverse or direct correlation, respectively, was found between B/I ratios of immunopurified basal TSH and the serum free T4 ($r = -0.7237$; $p<0.01$), T4 ($4 = -0.6650$; $p<0.05$), T3 ($r = -0.6382$; $p<0.05$) and TSH ($r = +0.8355$; $p<0.001$) concentrations measured by radioimmunoassay from the normal and hypothyroid individuals. When B/I ratios were evaluated for immunopurified TSH from serum before and at 20

min, 45+60 min, and 120+180 min after acute stimulation of TSH release by TRH in 3 primary hypothyroid patients, no significant variation of TSH bioactivity was found in each individual at various time points, despite major changes in serum concentration. All patients with TSH-secreting pituitary tumors had elevated basal serum TSH levels before pituitary surgery (range 9 to 568 μU/ml) and were at that stage chemically and clinically hyperthyroid. Their B/I ratios varied before surgery from 0.50 to 1.96. In two of these patients the elevated B/I ratios decreased to the range of euthyroid normals after successful pituitary surgery that rendered them biochemically and clinically euthyroid. In a third patient, however, reevaluated after non-successful pituitary surgery, the B/I ratio remained high and the patient continued to show all classical signs of ongoing hyperthyroid disease. In summary: (1) the present investigation shows an inverse relationship between the metabolic status of an individual in terms of circulating thyroid hormone levels and the biological potency of the circulating TSH, a physiological feed-back mechanism not earlier described; (2) in TSH-secreting tumors, where this feed-back mechanism is disturbed, repetitive determinations of B/I ratios seem to serve as a marker of the activity of the disease; (3) TSH B/I ratio determinations should probably prove useful in screening for mild cases of TSH-secreting pituitary tumors.

. . . . P. A. Dahlberg, M. Nissim, B. D. Weintraub

I. Enzymatic deglycosylation of thyrotropin

Previous studies of the functional role of the carbohydrate moieties in TSH from our and other laboratories have all used chemical methods to deglycosylate the hormone. These methods involve the use of hydrogen fluoride which leads to incomplete and heterogeneous removal of the carbohydrate and may affect the TSH apoprotein. We investigated the ability of two enzymes, peptide N-glycosidase F (PNGase F) and endo-β-N-acetylglucosaminidase F (Endo F), to deglycosylate microgram quantities of bovine TSH and its subunits under nondenaturing conditions. The products were analyzed by gel electrophoresis, gel permeation HPLC, amino acid and amino sugar content. It was shown that one oligosaccharide chain could be selectively removed from TSH-α subunit by PNGase F, and all the oligosaccharide chains from both subunits could be removed by Endo F. These methods of enzymatic deglycosylation are currently being used to study the functional role of each of the N-linked carbohydrate chains in TSH and other glycoprotein hormones.

. . . . K. O. Lee, N.Gesundheit, B. D. Weintraub

J. Effects of thyrotropin-releasing hormone (TRH) on TSH subunit messenger RNA levels.

We have investigated the in vivo effects of TRH on thyrotroph function and number in 4 week old male Sprague-Dawley rats. The pituitary contents of DNA, immunoreactive TSH, and mRNA levels for TSH subunits were determined. Serum levels for TSH, free T4, and T3 were measured, and the number of thyrotrophs was estimated as the number of pituitary cells that were positively immunostained for TSH. Our data showed that, in the presence or absence of thyroid hormones and/or hypothalamic

influence, TRH, given as a single injection or a continuous infusion, fails to increase the rat pituitary levels of mRNA for both TSH subunits; therefore, TRH regulates TSH production, most likely, at the posttranslational level of hormone synthesis. We have also compared the effects of TRH on pituitary tissue in situ (sella) and after its transplantation under the renal capsule. We have found that, like in sellar pituitary, in transplanted tissue TRH does not significantly affect either the number of thyrotrophs or their ability to synthesize TSH subunit mRNAs. However, TRH is required to maintain released TSH in circulation, since serum TSH levels were low in the absence of this hormone.

We measured TSH subunit mRNA levels in 5 week old normal male rats kept under standard artificial fluorescent lighting on a 12:12 hour, light:dark cycle. We found no significant diurnal variation in the mRNA levels. TSH immunoreactivity has recently been detected in human lymphocytes and it has been proposed that various neuroendocrine peptides mediate neuroimmunoregulation. Therefore, in collaboration with John Mounts of the ARD Branch, Northern transfers of RNA from mouse and human lymphocytes, normal and lymphodysplastic, were screened for the presence of α or TSHβ mRNAs. No signals of the appropriate hybridization characteristics or size were found.

Currently we are examining the effects of TRH, cyclic nucleotides and other factors on TSH subunit messenger RNA levels in dispersed pituitary cells in primary culture.

. . . . S. S. Lippman, S. Amr, I. Calvert, B. D. Weintraub

II. NEUROENDOCRINE PEPTIDES: BIOSYNTHESIS, FOLDING AND FUNCTION

A. Molecular mechanisms in neuroendocrine peptide and protein pathways

Molecular mechanisms underlying the neuroendocrine occurrence, biosynthesis, molecular characteristics and function of neuroendocrine peptides and proteins are being studied, with current emphasis on the neuropeptide hormones oxytocin and vasopressin and associated neurophysins (NP's). An hypothesis continues to be examined that biosynthetic precursors of the neurohypophysial hormones adopt a defined conformational organization upon completion of translation and that this organization helps regulate the production of active peptides produced in neuroendocrine pathways which make the precursors. A method devised to produce biosynthetic precursors, and ultimately sequence-modeled and site-specific mutants, has been used to prepare semisynthetic oxytocin/neurophysin I and Arg 8 vasopressin/neurophysin II precursor analogs. Evaluation of structural characteristics of the semisynthetic precursors shows that the precursors are well-ordered, folded molecules which can form self-associated species. The latter are likely to be the prevailing forms in neurosecretory granules in which enzymatic processing occurs. Evaluation of the impact of these characteristics on enzymatic processing also continues, by comparing rates and products of processing of intact precursors to these properties for synthetic fragments containing processing sites. Separately, evaluation has been extended of the relationship between occurrence of the hormone/NP

neuroendocrine system in the ovary versus that in hypothalamo-neurophysial system. The relationships of ovarian molecular species to those produced in the hypothalamo-neurohypophysial pathway are being studied. The data are being used to help define the relationship between molecular mechanisms which occur in different neuroendocrine sites. In addition to neurophysin and oxytocin, a newly identified neurophysin-binding species has been found in both sites and is currently being characterized.

. . . . I. M. Chaiken, S. Ando, G. Fassina, X.-F. Chen

B. Mechanisms and engineering of peptide/protein recognition, assembly, function

Principles which govern surface recognition, intra- and intermolecular assembly and function of peptides and proteins are being studied. Molecular recognition by peptides and proteins underlies essentially all biological functions of these substances, emphasizing the importance of understanding surface organization and dynamics in determining molecular order and function. This issue has been addressed with the neurohypophysial hormones oxytocin and vasopressin and associated neurophysins, which form cooperative peptide-protein complexes that act as storage forms for the polypeptides in neurosecretory granules, and the hormone-neurophysin precursors which also appear to self-associate into forms likely to exist in granules before processing. The nature and structural interrelationships between the self-association and hormone binding surfaces in neurophysins that give rise to cooperative complexes have been studied, using natural hormones and hormone mutants obtained by chemical synthesis. In addition, sequence-variant mutants of precursor have been prepared by semisynthesis and their interaction properties are being studied. Separately, underlying principles which determine surface recognition and consequent molecular order are being evaluated by studying the effect of synthetic sequence mutation on the peptide-protein assembly of semisynthetic ribonuclease-S, using high resolution structure of a modeled semisynthetic ribonuclease-S as a starting point. The data are being used to examine rules of protein self-assembly and to establish general guidelines for protein engineering. In addition, protein engineering by recombinant DNA methods is being used to examine the general usefulness of site-specific random sequence mutation, with initial experiments being carried out using lambda repressor. Eventually, correlation of helical packing, domain assembly, flexibility and proteolysis should be useful to better understand the regulation of enzymatic processing of endocrine "multi-domain" precursors.

. . . . I. M. Chaiken, G. Fassina, S. Ando, Y. Shai

C. Biorecognition and biorecognition methodology

Bioaffinity methods and principles are being developed which can be used for characterizing functional interaction properties, including multimolecular assembly, of biological macromolecules and to design polypeptides de novo which recognize protein surfaces. A major study has been designed to evaluate the potential to adapt bioaffinity chromatography to extant high performance liquid chromatography technology. Silica-

based matrices are being used to measure protein-protein, peptide-protein, and peptide-peptide interactions, for neuroendocrine peptides and proteins and their precursors as well as well-understood "model" proteins. Analytical high performance affinity chromatography also is being used as an evaluative tool to design and chemically synthesize peptides de novo which can recognize protein surfaces specifically and ultimately be used for protein isolation and for diagnostic characterization of macromolecular recognition properties. Overall analytical high performance affinity chromatographic methods which result from this study provide potentially important analytical biochemistry tools both for characterizing basic properties of macromolecules and for microscale molecular profiling and diagnosis.

. . . . I. M. Chaiken, G. Fassina, Y. Shai

III. HORMONES AND RECEPTORS

A. Insulin-like Growth Factors (Somatomedins): Biosynthesis and Action

We have continued our study of the insulin-like growth factor/somatomedin, rat IGF-II, a polypeptide synthesized by a cloned line of rat liver cells (BRL-3A). During the past year, progress has been made in the following areas: (1) determination of the structure of pre-pro-rIGF-II and pro-rIGF-II by molecular cloning and radiosequencing; (2) demonstration that IGF-II mRNA levels are high in fetal and neonatal rat liver and decrease in adult tissues, suggesting that the developmental regulation of IGF-II may occur at the level of transcription; (3) demonstration that the major IGF-II RNA is 4 kilobases, considerably larger than the functional 1.2 kb IGF-II RNA used to establish our cDNA library; (4) demonstration that the 4 kb IGF-II RNA does not direct translation of pre-pro-rIGF-II, and may require further processing to become activated; (5) demonstration that growth hormone regulates the abundance of IGF-I mRNA in adult rat liver, suggesting possible transcriptional regulation; (6) demonstration that the tyrosine kinase activity of the β-subunit of the type I IGF receptor closely resembles that of the insulin receptor, suggesting that the homologies between the two receptors extends to their kinase domains; (7) development of antibodies to the Mr 40,000 IGF carrier protein in neonatal rat serum, and demonstrating that it is immunologically distinct from the Mr 150,000 carrier protein in adult rat serum; (8) demonstration that chemically synthesized hybrid molecules containing the A-chain of insulin and the B-domain of IGF-I bind with enhanced affinity to IGF carrier proteins; and (9) demonstration using these hybrid molecules that IGF carrier proteins synthesized by cultured human fibroblasts modulate the binding of IGF-I to IGF receptors on cell monolayers.

. . . . M. M. Rechler, J. A. Romanus, D. E. Graham, L. Tseng, J.-F. Wang, A. L. Brown, D. R. Clemmons

IV. STUDIES OF THE MECHANISM OF THE INSULIN ACTION AND ITS PERTURBATION IN ALTERED METABOLIC STATES

A. Insulin-Cell Interaction

The biosynthesis of the insulin receptor has been studied in primary cultures of isolated rat adipose cells. The results suggest that insulin receptors are synthesized through the formation and processing of a 190K precursor in the endoplasmic reticulum and Golgi followed by insertion of the mature 135K and 95K subunits into the plasma membrane.

. . . . I. A. Simpson, T. M. Weber, H. G. Joost, S. W. Cushman

B. Insulin's Regulation of Glucose Transport

The biochemical mechanism of insulin action on glucose transport in the rat adipose cell has been studied using a photochemical crosslinking agent to covalently bind [^{3}H]cytochalasin B to the glucose transporter. The data demonstrate that: 1) there is a heterogeneity of glucose transporter species in the intracellular pool while the plasma membrane transporters are more uniform in structure. 2) The pH 5.6 glucose transporter isoform is translocated by insulin from the low-density microsomes to the plasma membrane but the pH 6.4 isoform is not sensitive to insulin. 3) Differential sensitivity of the glucose transporter isoforms to neuraminidase suggests that the heterogeneity is at least partially due to differences in the glycosylation state. 3T3-L1 fibroblasts differentiate in culture to resemble adipose cells both morphologically and biochemically. The number of glucose transporters has been measured in subcellular membrane fractions from these cells during differentiation. The data suggest that the glucose transporter undergoes differential processing and that functional, insulin-responsive glucose transporters may be different from the insulin-insensitive (basal) glucose transporter. The effect of glucose (Glc) deprivation (starvation) on hexose transporter (GT) polypeptide(s) (pp) was studied in 3T3-C2 murine fibroblasts. The results suggest that the accumulation of total GT pp induced by Glc deprivation is the result of specialized and sensitive adaptation. The GT pp synthesized during chronic Glc deprivation has an M_r of 42000; fed cells synthesize a M_r 55000 GT pp. Neither the level of *in vitro* translatable GT mRNA nor the rate of GT pp synthesis are increased by Glc deprivation. It is likely, therefore, that the accumulation of GT pp during Glc deprivation is the result of decreased degradation of GT pp. In a preliminary series of experiments, insulin appears to stimulate glucose transport in isolated human adipose cells by a translocation mechanism similar to that observed in rat adipose cells and diaphragm.

. . . . S. W. Cushman, I. A. Simpson, B. B. Kahn, K. C. Appell, H. G. Joost, D. L. Baly, T. M. Weber, M. J. Zarnowski, D. R. Yver.

D. Alterations in Insulin's Action in Insulin-Dependent Diabetes Mellitus

The effects of insulin therapy on the glucose transport response to insulin in adipocytes from stretozotocin diabetic rats have been

examined. The results suggest that insulin therapy produces markedly hyperresponsive insulin-stimulated adipocyte glucose transport but only in part by increasing intracellular glucose transporters and insulin-stimulated glucose transporter translocation to the plasma membrane. The remaining hyperresponsiveness appears to be due to concurrently augmented glucose transporter intrinsic activity.

. . . . B. B. Kahn, S. W. Cushman

E. Alterations in Insulin's Action with Chronic Hyperinsulinemia

The effects of chronic insulin administration on the metabolism of isolated rat adipose cells have been studied. The results suggest that chronic hyperinsulinemia increases insulin binding and the capacity of rat adipose cells to transport and metabolize glucose without changing the cells' sensitivity to insulin. The mechanism of increased insulin-stimulated glucose transport in adipocytes from chronically hyperinsulinemic rats has also been examined. These results suggest that chronic hyperinsulinemia in the rat enhances insulin's stimulatory action on glucose transport in adipocytes by increasing the intracellular pool of glucose transporters through a generalized effect on the net synthesis of intracellular protein.

. . . . B. B. Kahn, S. W. Cushman

F. Insulin's Regulation of Hormone Binding

A comparison of insulin's effects on glucose transport and cell surface IGF-II receptors has been undertaken in rat adipose cells using 3-O-methylglucose transport as a measure of glucose transport activity and Scatchard analysis of IGF-II binding in the presence of KCN to determine cell surface IGF-II receptor number. These results demonstrate that while the characteristics of the stimulatory action of insulin on glucose transport activity and cell surface IGF-II receptor number are qualitatively similar, quantitative differences are clearly demonstrable which suggest that the subcellular cycling of these two integral membrane proteins occurs by distinct processes. The effects of adenosine, isoproterenol, and glucose have now been examined on both steady state insulin responsiveness and sensitivity in this cell type prepared in the presence of saturating adenosine (200 nM). The results show that the stimulatory effect of insulin on IGF-II binding to rat adipose cells is modulated not only by counterregulatory hormones, but also by glucose, a major substrate of insulin action.

. . . . K. C. Appell, I. A. Simpson, S. W. Cushman, M. J. Zarnowski, B. B. Kahn, M. M. Rechler

G. Counterregulation of Insulin's Action by Catecholamines

The modulation of insulin-stimulated glucose transport activity in rat adipose cells by ligands for receptors (R) that mediate stimulation (R_s; lipolytic) or inhibition (R_i; antilipolytic) of adenylate cyclase has been examined. The results suggest that 1) R_i- and R_s-mediated effects on glucose transport are independent of changes in cAMP, 2) these cAMP-independent effects are mediated by GTP-binding proteins, N_i and

N_s, and 3) R_i and R_s ligands modulate the intrinsic activity of the glucose transporter in the plasma membrane. The mechanism of modulation of insulin-stimulated glucose transport activity in isolated rat adipose cells by lipolytic and antilipolytic agents has been further examined by measuring glucose transport activity in plasma membranes. The data indicate that modifications of glucose transport activity produced by lipolytic and antilipolytic agents in intact adipose cells can be fully retained in plasma membranes isolated under appropriate conditions, further supporting the concept that the effects of these agents occur through a modification of glucose transporter intrinsic activity. The effects of β-adrenergic stimulation and different analogues of cAMP on insulin-stimulated IGF-II binding have also been studied. The results indicate that β-adrenergic stimulation and high levels of cAMP markedly impair both sensitivity and responsiveness to insulin suggesting an antagonistic effect on insulin's signalling mechanism. Furthermore, adenosine appears to exert a potent modulating effect through N_i, while activation of phosphodiesterase by insulin appears to play a crucial role for the expression of insulin action under conditions of elevated cAMP levels.

. . . . I. A. Simpson, S. W. Cushman, T. M. Weber, K. C. Appell, M. J. Zarnowski

H. Alterations in Insulin's Action with Fasting/Refeeding

Rapid alterations in glucose transport and metabolism have been shown in rat adipose cells after fasting and refeeding. The mechanism for this was examined in rats fasted for 48 h and sacrificed $\pm$ 6 d of refeeding. The results suggest that insulin resistance at the glucose transport level induced by fasting is due to a depletion of intracellular glucose transporters. In contrast, the hyperresponsive insulin-stimulated glucose transport activity associated with refeeding is not totally accounted for by a change in the number of glucose transporters and may also involve modulation of glucose transporter intrinsic activity.

. . . . B. B. Kahn, S. W. Cushman

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 55000-14 MCNE

PERIOD COVERED: October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Biosynthesis and Glycosylation of Thyrotropin

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	B. D. Weintraub	Chief	MCNEB, NIDDK
Others:	B. S. Stannard	Biologist	MCNEB, NIDDK
	T. Taylor	Guest Researcher	MCNEB, NIDDK
	N. Gesundheit	Medical Staff Fellow	MCNEB, NIDDK
	P. W. Gyves	Guest Researcher	MCNEB, NIDDK
	J. B. Butler	Medical Staff Fellow	MCNEB, NIDDK

COOPERATING UNITS (if any)

Faculty of Medicine, University of Marseille, Marseille, France (C. Ronin)

LAB/BRANCH
Molecular, Cellular and Nutritional Endocrinology Branch

SECTION
Molecular Regulation and Neuroendocrinology Section

INSTITUTE AND LOCATION
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SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Formerly Project No. Z01 AM 55000-13 MCNE

Thyrotropin biosynthesis and carbohydrate processing has been studied under various physiologic conditions and in the presence of various carbohydrate processing inhibitors. Deoxynojirimycin, which inhibits glucosidase action and leads to accumulation of glucosylated precursors, caused inhibition of thyroropin secretion but no effects on the secretion of nonglycoprotein hormones. Thyroid hormone and thyrotropin-releasing hormone (TRH) caused differential effects on thyrotropin apoprotein and carbohydrate synthesis and secretion. TSH carbohydrate structure is heterogeneous, and thyrotropin-releasing hormone caused a specific increase in synthesis and secretion of biantennary complex forms. Patients with invasive thyrotropin-secreting pituitary tumors may secrete TSH with more branched carbohydrate structure. The carbohydrate structure of TSH changes during neonatal development and is altered by paraventricular nuclear lesions in rats causing hypothalamic hypothyroidism. The effects of TRH on TSH carbohydrate structure are specific and not induced by non-specific secretagogues such as potassium.

PHS 6040 (Rev. 1/84)

GPO 814-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 55001-10 MCNE

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regulation and Action of Thyrotropin

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. D. Weintraub	Chief	MCNEB, NIDDK
Others:	K. O. Lee	Visiting Fellow	MCNEB, NIDDK
	P. A. Dahlberg	Visiting Fellow	MCNEB, NIDDK
	M. Nissim	Guest Researcher	MCNEB, NIDDK
	S. Amr	Visiting Associate	MCNEB, NIDDK

COOPERATING UNITS *(if any)*
University of Milan, Italy; University of Lisbon, Portugal; Karolinska Institute, Sweden.

LAB/BRANCH
Molecular, Cellular and Nutritional Endocrinology Branch

SECTION
Molecular Regulation and Neuroendocrinology Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.9	2.7	.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Formerly Project No. Z01 AM 55001-09 MCNE

A large number of patients with both neoplastic and non-neoplastic inappropriate secretion of thyrotropin as well as patients with various degrees of primary hypothyroidism have been evaluated for TSH bioactivity. There is an inverse relationship between the metabolic status of an individual in terms of circulating hormone levels and the biological potency of the TSH. Moreover, patients with TSH-producing pituitary tumors show an increased bioactivity which may be an early marker for the disease. Enzymatic deglycosylation of TSH with peptide N-glycosidase F and endo-β-N-acetylglucosaminidase F can be achieved under non-denaturing conditions and will permit analysis of the role of each carbohydrate chain in TSH action.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 55002-06 MCNE

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Biology of Glycoprotein Hormones

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. D. Weintraub	Chief	MCNEB, NIDDK
Others:	I. Calvert	Chemist	MCNEB, NIDDK
	S. S. Lippman	Medical Staff Fellow	MCNEB, NIDDK
	S. Amr	Visiting Associate	MCNEB, NIDDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Molecular, Cellular and Nutritional Endocrinology Branch

SECTION

Molecular Regulation and Neuroendocrinology Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.3	2.1	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Formerly Project No. Z01 AM 55002-05 MCNE

The effects of thyrotropin-releasing hormone (TRH) on thyrotropin (TSH) subunit messenger RNA levels has been examined in various in vivo animal models. Despite major effects of TRH on TSH release, there were no effects on TSHα or β messenger RNA levels. Similarly, there were no effects of TRH on thyrotroph number. Thus factors other than TRH are responsible for the stimulation of TSH messenger RNA and thyrotroph growth.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 55003-13 MCNE

PERIOD COVERED: October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Molecular Mechanisms in Neuroendocrine Peptide and Protein Pathways

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Irwin M. Chaiken	Research Chemist	MCNEB, NIDDK
Others:	Shoji Ando	Visiting Fellow	MCNEB, NIDDK
	Giorgio Fassina	Visiting Fellow	MCNEB, NIDDK
	Xiu-Fang Chen	Guest Researcher	MCNEB, NIDDK

COOPERATING UNITS *(if any)*: Neurosciences Department, Johns Hopkins Medical School, Baltimore, MD; Postgraduate School of Obstetrics and Gynaecology, University of Auckland, New Zealand.

LAB/BRANCH: Molecular, Cellular and Nutritional Endocrinology Branch

SECTION: Molecular Regulation and Neuroendocrinology Section

INSTITUTE AND LOCATION: NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	1.1	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Formerly Project No. Z01 AM 25,007-12 MCNE

Molecular mechanisms underlying the neuroendocrine occurrence, biosynthesis, molecular characteristics and function of neuroendocrine peptides and proteins are being studied, with current emphasis on the neuropeptide hormones oxytocin and vasopressin and associated neurophysins (NP's). An hypothesis continues to be examined that biosynthetic precursors of the neurohypophysial hormones adopt a defined conformational organization upon completion of translation and that this organization helps regulate the production of active peptides produced in neuroendocrine pathways which make the precursors. A method devised to produce biosynthetic precursors, and ultimately sequence-modeled and site-specific mutants, has been used to prepare semisynthetic oxytocin/neurophysin I and Arg 8 vasopressin/neurophysin II precursor analogs. Evaluation of structural characteristics of the semisynthetic precursors shows that the precursors are well-ordered, folded molecules which can form self-associated species. The latter are likely to be the prevailing forms in neurosecretory granules in which enzymatic processing occurs. Evaluation of the impact of these characteristics on enzymatic processing also continues, by comparing rates and products of processing of intact precursors to these properties for synthetic fragments containing processing sites. Separately, evaluation has been extended of the relationship between occurrence of the hormone/NP neuroendocrine system in the ovary versus that in hypothalamo-neurophysial system. The relationships of ovarian molecular species to those produced in the hypothalamo-neurohypophysial pathway are being studied. The data are being used to help define the relationship between molecular mechanisms which occur in different neuroendocrine sites. In addition to neurophysin and oxytocin, a newly identified neurophysin-binding species has been found in both sites and is currently being characterized.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 55004-16 MCNE

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanisms and Engineering of Peptide/Protein Recognition, Assembly, Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Irwin M. Chaiken	Research Chemist	MCNEB, NIDDK
Others:	Giorgio Fassina	Visiting Fellow	MCNEB, NIDDK
	Shoji Ando	Visiting Fellow	MCNEB, NIDDK
	Yechiel Shai	Guest Researcher	MCNEB, NIDDK

COOPERATING UNITS *(if any)* Inst. of Organic Chem., Univ. of Padova, Italy; Mass. General Hosp., Harvard Univ. Med. School; Chem. Dept., Nankai Univ. Tianjin, Peoples Republic of China; Inst. of Organic Chem. and Biochem., Czechoslovak Academy of Sciences, Prague, CSSR; Biophysics Dept., Johns Hopkins Medical School, Baltimore, MD

LAB/BRANCH

Molecular, Cellular and Nutritional Endocrinology Branch

SECTION

Molecular Regulation and Neuroendocrinology Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.6	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Formerly Project No. Z01 AM 25,004-15 MCNE

Principles which govern surface recognition, intra- and intermolecular assembly and function of peptides and proteins are being studied. Molecular recognition by peptides and proteins underlies essentially all biological functions of these substances, emphasizing the importance of understanding surface organization and dynamics in determining molecular order and function. This issue has been addressed with the neurohypophysial hormones oxytocin and vasopressin and associated neurophysins, which form cooperative peptide-protein complexes that act as storage forms for the polypeptides in neurosecretory granules, and the hormone-neurophysin precursors which also appear to self-associate into forms likely to exist in granules before processing. The nature and structural interrelationships between the self-association and hormone binding surfaces in neurophysins that give rise to cooperative complexes have been studied, using natural hormones and hormone mutants obtained by chemical synthesis. In addition, sequence-variant mutants of precursor have been prepared by semisynthesis and their interaction properties are being studied. Separately, underlying principles which determine surface recognition and consequent molecular order are being evaluated by studying the effect of synthetic sequence mutation on the peptide-protein assembly of semisynthetic ribonuclease-S, using high resolution structure of a modeled semisynthetic ribonuclease-S as a starting point. The data are being used to examine rules of protein self-assembly and to establish general guidelines for protein engineering. In addition, protein engineering by recombinant DNA methods is being used to examine the general usefulness of site-specific random sequence mutation, with initial experiments being carried out using lambda repressor. Eventually, correlation of helical packing, domain assembly, flexibility and proteolysis should be useful to better understand the regulation of enzymatic processing of endocrine "multi-domain" precursors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 55005-16 MCNE

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biorecognition and Biorecognition Methodology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Irwin M. Chaiken	Research Chemist	MCNEB, NIDDK
Others:	Giorgio Fassina	Visiting Fellow	MCNEB, NIDDK
	Yechiel Shai	Guest Researcher	MCNEB, NIDDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Molecular, Cellular and Nutritional Endocrinology Branch

SECTION

Molecular Regulation and Neuroendocrinology Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.4	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Formerly Project No. Z01 AM 15,003-15 MCNE

Bioaffinity methods and principles are being developed which can be used for characterizing functional interaction properties, including multi-molecular assembly, of biological macromolecules and to design polypeptides de novo which recognize protein surfaces. A major study has been designed to evaluate the potential to adapt bioaffinity chromatography to extant high performance liquid chromatography technology. Silica-based matrices are being used to measure protein-protein, peptide-protein, and peptide-peptide interactions, for neuro-endocrine peptides and proteins and their precursors as well as well-understood "model" proteins. Analytical high performance affinity chromatography also is being used as an evaluative tool to design and chemically synthesize peptides de novo which can recognize protein surfaces specifically and ultimately be used for protein isolation and for diagnostic characterization of macromolecular recognition properties. Overall analytical high performance affinity chromatographic methods which result from this study provide potentially important analytical biochemistry tools both for characterizing basic properties of macromolecules and for microscale molecular profiling and diagnosis.

PHS 6040 (Rev. 1/84) GPO 914-818

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 55006-13 MCNE

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Insulin-like Growth Factors (Somatomedins): Biosynthesis and Action

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. M. Rechler	Chief, GD Section	MCNEB, NIDDK
Others:	J. A. Romanus	Biologist	MCNEB, NIDDK
	D. E. Graham	Expert	MCNEB, NIDDK
	L. Tseng	Chemist	MCNEB, NIDDK
	J.-F. Wang	Visiting Fellow	MCNEB, NIDDK
	A. L. Brown	Staff Fellow	MCNEB, NIDDK
	D.R. Clemmons	Guest Researcher	MCNEB, NIDDK

COOPERATING UNITS *(if any)*
DB, NIDDK (C. Roberts), MB NCI (S.P. Nissley); Univ. Naples, Italy (C.B. Bruni, R. Frunzio); Mt. Sinai Sch. Med., CUNY, NY, (G.T. Burke, P. G. Katsoyannis, S.P. Joshi)

LAB/BRANCH
Molecular, Cellular and Nutritional Endocrinology Branch

SECTION
Growth and Development Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.5	4.5	2.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Formerly Project No. Z01 AM 55006-12 MCNEB
We have continued our study of the insulin-like growth factor/somatomedin, rat IGF-II, a polypeptide synthesized by a cloned line of rat liver cells (BRL-3A). During the past year, progress has been made in the following areas: (1) determination of the structure of pre-pro-rIGF-II and pro-rIGF-II by molecular cloning and radiosequencing; (2) demonstration that IGF-II mRNA levels are high in fetal and neonatal rat liver and decrease in adult tissues, suggesting that the developmental regulation of IGF-II may occur at the level of transcription; (3) demonstration that the major IGF-II RNA is 4 kilobases, considerably larger than the functional 1.2 kb IGF-II RNA used to establish our cDNA library; (4) demonstration that the 4 kb IGF-II RNA does not direct translation of pre-pro-rIGF-II, and may require further processing to become activated; (5) demonstration that growth hormone regulates the abundance of IGF-I mRNA in adult rat liver, suggesting possible transcriptional regulation; (6) demonstration that the tyrosine kinase activity of the β-subunit of the type I IGF receptor closely resembles that of the insulin receptor, suggesting that the homologies between the two receptors extends to their kinase domains; (7) development of antibodies to the Mr 40,000 IGF carrier protein in neonatal rat serum, and demonstrating that it is immunologically distinct from the Mr 150,000 carrier protein in adult rat serum; (8) demonstration that chemically synthesized hybrid molecules containing the A-chain of insulin and the B-domain of IGF-I bind with enhanced affinity to IGF carrier proteins; and (9) demonstration using these hybrid molecules that IGF carrier proteins synthesized by cultured human fibroblasts modulate the binding of IGF-I to IGF receptors on cell monolayers.

GPO 914-916

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 55007-08 MCNE

PERIOD COVERED: October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Insulin-Cell Interaction

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	I. A. Simpson	Visiting Scientist	MCNEB, NIDDK
Others:	T. M. Weber	Staff Fellow	MCNEB, NIDDK
	H. G. Joost	Guest Worker	MCNEB, NIDDK
	S. W. Cushman	Chief, EDMNS	MCNEB, NIDDK

COOPERATING UNITS (if any)

DB/NIDDK (J. A. Hedo, S. DiPaolo, G. Grunberger). Department of Medicine, University of Gothenburg, Gothenburg, Sweden (U. Smith).

LAB/BRANCH: Molecular, Cellular and Nutritional Endocrinology Branch

SECTION: Experimental Diabetes, Metabolism and Nutrition Section

INSTITUTE AND LOCATION: NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.4	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Formerly Project No. Z01 AM 55007-07 MCNE

The biosynthesis of the insulin receptor has been studied in primary cultures of isolated rat adipose cells. The results suggest that insulin receptors are synthesized through the formation and processing of a 190K precursor in the endoplasmic reticulum and Golgi followed by insertion of the mature 135K and 95K subunits into the plasma membrane.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 55008-08 MCNE

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Insulin's Regulation of Glucose Transport

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S. W. Cushman	Chief, EDMNS	MCNEB, NIDDK
Others:	I. A. Simpson	Visiting Scientist	MCNEB, NIDDK
	B. B. Kahn	Medical Staff Fellow	MCNEB, NIDDK
	K. C. Appell	Staff Fellow	MCNEB, NIDDK
	H. G. Joost	Guest Worker	MCNEB, NIDDK
	D. L. Baly	IPA	MCNEB, NIDDK
	T. M. Weber	Staff Fellow	MCNEB, NIDDK
	M. J. Zarnowski	Biologist	MCNEB, NIDDK
	D. R. Yver	Chemist	MCNEB, NIDDK

COOPERATING UNITS *(if any)* Program of Molecular Biology and Virology, Memorial Sloan-Kettering Cancer Center, New York, NY (H. C. Haspel, O. M. Rosen); Department of Medicine, University of California at San Diego Medical School, La Jolla, CA (R. Horuk, J. M. Olefsky); Department of Medicine, University of Gothenburg, Gothenburg, Sweden (U. Smith); (continued)

LAB/BRANCH

Molecular, Cellular and Nutritional Endocrinology Branch

SECTION

Experimental Diabetes, Metabolism and Nutrition Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.7	4.7	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Formerly Project No. Z01 AM 55008-07 MCNE

The biochemical mechanism of insulin action on glucose transport in the rat adipose cell has been studied using a photochemical crosslinking agent to covalently bind [^{3}H]cytochalasin B to the glucose transporter. The data demonstrate that: 1) there is a heterogeneity of glucose transporter species in the intracellular pool while the plasma membrane transporters are more uniform in structure. 2) The pH 5.6 glucose transporter isoform is translocated by insulin from the low-density microsomes to the plasma membrane but the pH 6.4 isoform is not sensitive to insulin. 3) Differential sensitivity of the glucose transporter isoforms to neuraminidase suggests that the heterogeneity is at least partially due to differences in the glycosylation state. 3T3-L1 fibroblasts differentiate in culture to resemble adipose cells both morphologically and biochemically. The number of glucose transporters has been measured in subcellular membrane fractions from these cells during differentiation. The data suggest that the glucose transporter undergoes differential processing and that functional, insulin-responsive glucose transporters may be different from the insulin-insensitive (basal) glucose transporter. The effect of glucose (Glc) deprivation (starvation) on hexose transporter (GT) polypeptide(s) (pp) was studied in 3T3-C2 murine fibroblasts. The results suggest that the accumulation of total GT pp induced by Glc deprivation is the result of specialized and sensitive adaptation. The GT pp synthesized during chronic Glc deprivation has an M_r of 42000; fed cells synthesize a M_r 55000 GT pp. Neither the level of *in vitro* translatable GT mRNA nor the rate of GT pp synthesis are increased by Glc deprivation. It is likely, therefore, that the accumulation of GT pp during Glc deprivation is the result of decreased degradation of GT pp. In a preliminary series of experiments, insulin appears to stimulate glucose transport in isolated human adipose cells by a translocation mechanism similar to that observed in rat adipose cells and diaphragm.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 AM 55009-08 MCNE

PERIOD COVERED: October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Alterations in Insulin's Action in Obesity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: S. W. Cushman Chief, EDMNS MCNEB, NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH: Molecular, Cellular and Nutritional Endocrinology Branch

SECTION: Experimental Diabetes, Metabolism and Nutrition

INSTITUTE AND LOCATION: NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Inactive.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 55010-04 MCNE

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Alterations in Insulin's Action in Insulin-Dependent Diabetes Mellitus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. B. Kahn	Medical Staff Fellow	MCNEB, NIDDK
Others:	S. W. Cushman	Chief, EDMNS	MCNEB, NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Molecular, Cellular and Nutritional Endocrinology Branch

SECTION

Experimental Diabetes, Metabolism and Nutrition Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Formerly Project No. Z01 AM 55010-03 MCNE

The effects of insulin therapy on the glucose transport response to insulin in adipocytes from stretozotocin diabetic rats have been examined. The results suggest that insulin therapy produces markedly hyperresponsive insulin-stimulated adipocyte glucose transport but only in part by increasing intracellular glucose transporters and insulin-stimulated glucose transporter translocation to the plasma membrane. The remaining hyperresponsiveness appears to be due to concurrently augmented glucose transporter intrinsic activity.

PHS 6040 (Rev. 1/84)

GPO 814-818

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 55011-04 MCNE

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Alterations in Insulin's Action with Chronic Hyperinsulinemia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. B. Kahn	Medical Staff Fellow	MCNEB, NIDDK
Others:	S. W. Cushman	Chief, EDMNS	MCNEB, NIDDK

COOPERATING UNITS *(if any)*

Metabolic Unit, Department of Medicine, University of Vermont College of Medicine, Burlington, VT (L.J. Wardzala, E. S. Horton).

LAB/BRANCH

Molecular, Cellular and Nutritional Endocrinology Branch

SECTION

Experimental Diabetes, Metabolism and Nutrition

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Formerly Project No. Z01 AM 55011-03 MCNE

The effects of chronic insulin administration on the metabolism of isolated rat adipose cells have been studied. The results suggest that chronic hyperinsulinemia increases insulin binding and the capacity of rat adipose cells to transport and metabolize glucose without changing the cells' sensitivity to insulin. The mechanism of increased insulin-stimulated glucose transport in adipocytes from chronically hyperinsulinemic rats has also been examined. These results suggest that chronic hyperinsulinemia in the rat enhances insulin's stimulatory action on glucose transport in adipocytes by increasing the intracellular pool of glucose transporters through a generalized effect on the net synthesis of intracellular protein.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 55012-04 MCNE

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Insulin's Regulation of Hormone Binding

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	K. C. Appell	Staff Fellow	MCNEB, NIDDK
Others:	I. A. Simpson	Visiting Scientist	MCNEB, NIDDK
	S. W. Cushman	Chief, EDMNS	MCNEB, NIDDK
	M. J. Zarnowski	Biologist	MCNEB, NIDDK
	B. B. Kahn	Medical Staff Fellow	MCNEB, NIDDK
	M. M. Rechler	Chief, GDS	MCNEB, NIDDK

COOPERATING UNITS *(if any)*

Department of Medicine, University of Gothenburg, Gothenburg, Sweden (U. Smith).

LAB/BRANCH

Molecular, Cellular and Nutritional Endocrinology Branch

SECTION

Experimental Diabetes, Metabolism and Nutrition Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Formerly Project No. Z01 AM 55012-03 MCNE

A comparison of insulin's effects on glucose transport and cell surface IGF-II receptors has been undertaken in rat adipose cells using 3-0-methylglucose transport as a measure of glucose transport activity and Scatchard analysis of IGF-II binding in the presence of KCN to determine cell surface IGF-II receptor number. These results demonstrate that while the characteristics of the stimulatory action of insulin on glucose transport activity and cell surface IGF-II receptor number are qualitatively similar, quantitative differences are clearly demonstrable which suggest that the subcellular cycling of these two integral membrane proteins occurs by distinct processes. The effects of adenosine, isoproterenol, and glucose have now been examined on both steady state insulin responsiveness and sensitivity in this cell type prepared in the presence of saturating adenosine (200 nM). The results show that the stimulatory effect of insulin on IGF-II binding to rat adipose cells is modulated not only by counter-regulatory hormones, but also by glucose, a major substrate of insulin action.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 55013-03 MCNE

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Counterregulation of Insulin's Action by Catecholamines

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H. G. Joost	Guest Worker	MCNEB, NIDDK
Others:	I. A. Simpson	Visiting Scientist	MCNEB, NIDDK
	S. W. Cushman	Chief, EDMNS	MCNEB, NIDDK
	T. M. Weber	Staff Fellow	MCNEB, NIDDK
	K. C. Appell	Staff Fellow	MCNEB, NIDDK
	M. J. Zarnowski	Biologist	MCNEB, NIDDK

COOPERATING UNITS *(if any)*

Department of Medicine, Univ. of Gothenburg, Gothenburg, Sweden (P. Lönnroth, U. Smith); Department of Biochemistry, The University of Newcastle upon Tyne, Newcastle upon Tyne, England (R. C. Honnor); LCDB/NIDDK (C. Londos).

LAB/BRANCH

Molecular, Cellular and Nutritional Endocrinology Branch

SECTION

Experimental Diabetes, Metabolism and Nutrition Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Formerly Project No. Z01 AM 55013-02 MCNE

The modulation of insulin-stimulated glucose transport activity in rat adipose cells by ligands for receptors (R) that mediate stimulation (R_s; lipolytic) or inhibition (R_i; antilipolytic) of adenylate cyclase has been examined. The results suggest that 1) R_i- and R_s-mediated effects on glucose transport are independent of changes in cAMP, 2) these cAMP-independent effects are mediated by GTP-binding proteins, N_i and N_s, and 3) R_i and R_s ligands modulate the intrinsic activity of the glucose transporter in the plasma membrane. The mechanism of modulation of insulin-stimulated glucose transport activity in isolated rat adipose cells by lipolytic and antilipolytic agents has been further examined by measuring glucose transport activity in plasma membranes. The data indicate that modifications of glucose transport activity produced by lipolytic and antilipolytic agents in intact adipose cells can be fully retained in plasma membranes isolated under appropriate conditions, further supporting the concept that the effects of these agents occur through a modification of glucose transporter intrinsic activity. The effects of β-adrenergic stimulation and different analogues of cAMP on insulin-stimulated IGF-II binding have also been studied. The results indicate that β-adrenergic stimulation and high levels of cAMP markedly impair both sensitivity and responsiveness to insulin suggesting an antagonistic effect on insulin's signalling mechanism. Furthermore, adenosine appears to exert a potent modulating effect through N_i, while activation of phosphodiesterase by insulin appears to play a crucial role for the expression of insulin action under conditions of elevated cAMP levels.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 55014-03 MCNE

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Alterations in Insulin's Action with Fasting/Refeeding

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. B. Kahn	Medical Staff Fellow	MCNEB, NIDDK
Others:	S. W. Cushman	Chief, EDMNS	MCNEB, NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Molecular, Cellular and Nutritional Endocrinology Branch

SECTION
Experimental Diabetes, Metabolism and Nutrition Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Formerly Project No. Z01 AM 55014-02 MCNE

Rapid alterations in glucose transport and metabolism have been shown in rat adipose cells after fasting and refeeding. The mechanism for this was examined in rats fasted for 48 h and sacrificed ± 6 d of refeeding. The results suggest that insulin resistance at the glucose transport level induced by fasting is due to a depletion of intracellular glucose transporters. In contrast, the hyperresponsive insulin-stimulated glucose transport activity associated with refeeding is not totally accounted for by a change in the number of glucose transporters and may also involve modulation of glucose transporter intrinsic activity.

PHS 6040 (Rev. 1/84)

GPO 914-918

ANNUAL REPORT OF THE LABORATORY OF STRUCTURAL BIOLOGY
NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

I. BIOLOGY OF COMPLEX CARBOHYDRATES

Cell surface carbohydrates change during development suggesting that they are probably involved in differentiation. These developmentally-regulated changes allow some antibodies directed against carbohydrates to discriminate among tissues, both normal and malignant. To obtain cell-specific monoclonal antibodies, mice and rats have been immunized with various cell types in many laboratories. Some of the antibodies derived from spleen cells of the immunized animals that have an apparent specificity for certain cells and developmental stages are directed against carbohydrates. The structures of over 100 of these carbohydrate antigens have been elucidated. Most of them are related to the human ABO and Lewis blood group antigens. The antibodies are being used to study changes in cell surface carbohydrates during development in hopes of providing insights into the functions of glycoconjugates. For example, antibody LeoMel 3 which is directed against the ganglioside GD3, specifically inhibits killing of melanoma cells by human anomalous killer cells. These killer cells differentiate from classical cytotoxic T lymphocytes and lyse freshly isolated human melanoma cells which are insensitive to natural killer cell-mediated lysis. It is likely that the killer cells recognize the sugar sequence of ganglioside GD3. Antibody LeoMel 3 is an IgM. Other IgM antibodies from the same fusion that bind melanoma cells do not inhibit killer cell-mediated lysis. Another antibody, 18B8, which is directed against the ganglioside GT3, reacts only with the inner and outer synaptic layers of developing chick retina. It is possible that the sugar sequence of ganglioside GT3 is involved in synapse formation possibly by binding to lectin-like molecules on neuronal cell surfaces.

.....Drs. V. Ginsburg, J. Magnani, S. Spitalnik, D. Roberts, C. Dubois, P. Spitalnik, S. Fukuta

II. METABOLISM AND ROLE OF POLYSACCHARIDE SULFATES

The discovery of a novel sulfatase of unusual specificity and the synthesis of isomeric glucosamine sulfates of known structure have led to the discovery that heparin contains a 3-O sulfated glucosamine residue which is essential for its role as an anticoagulant. The enzyme, which has been partially purified from pooled human urine is present as several differently charged forms. Many polyanions, including heparin, induce allosteric changes in the structure of hemoglobin. In a study of the effects on hemoglobin of polyanions of controlled size, highly sulfated trehalose and stachyose have been prepared. These compounds bind with high affinity to hemoglobin-S and strongly decrease its affinity for oxygen.

.....Dr. I. Leder

III. EXPRESSION AND FUNCTION OF BACTERIAL CELL SURFACE COMPONENTS

The E. coli K1 capsule is involved in pathogenic processes such as virulence and invasiveness. Expression of K1 capsule synthesis on the bacterial cell surface requires porin proteins in the outer membrane. A specific porin, protein K, found in all K1 encapsulated strains, supports the more rapid expression of K1 capsule as compared to other porin proteins tested. There was, however, no increase in the steady state amount of K1 capsule in strains containing protein K. Thus, protein K may be more efficient in the organization of a putative outer membrane complex involved in the extracellular transport on the K1 capsule. Bacteria are ingested and degraded by macrophages. However, certain bacterial components such as LPS may persist for several days within the macrophage after phagacytosis. Outer membrane proteins also persist. These reflect this association. The O-PS portion of LPS in the outer membrane of Salmonellae regulates the extent of deposition of the complement component C3b and hence regulates the rate of phagacytosis of these cells by macrophages. Relatively minor changes in the structure of different O-PS found in various strains may have profound effects on the clearance of these cells and thus alter their virulence in mice. The O-PS functions in this process by influencing the activation of complement by the alternate pathway by altering the binding of factor B. and hence the amplification of deposition of C3b.

.....Drs. J. Foulds, V. Jiminez, S. Schieber, S. Stickley

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 57000-21 LSB
Formerly
Z01 AM 23630-21 LBP

PERIOD COVERED
October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biology of Complex Carbohydrates

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Victor Ginsburg, Ph.D.	Chief, LSB	LSB	NIDDK
Others:	John L. Magnani, Ph.D.	Senior Staff Fellow	LSB	NIDDK
	Steven L. Spitalnik, M.D.	Medical Staff Fellow	LSB	NIDDK
	David D. Roberts, Ph.D.	Staff Fellow	LSB	NIDDK
	Catherine-Marie Dubois, Ph.D.	Visiting Fellow	LSB	NIDDK
	Patrice F. Spitalnik, M.D.	Guest Researcher	LSB	NIDDK
	Shinji Fukuta, M.D.	Guest Researcher	LSB	NIDDK

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Structural Biology

SECTION
Section on Biochemistry

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.0	5.0	2.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Cell surface carbohydrates change during development suggesting that they are probably involved in differentiation. These developmentally-regulated changes allow some antibodies directed against carbohydrates to discriminate among tissues, both normal and malignant. To obtain cell-specific monoclonal antibodies, mice and rats have been immunized with various cell types in many laboratories. Some of the antibodies derived from spleen cells of the immunized animals that have an apparent specificity for certain cells and developmental stages are directed against carbohydrates. The structures of over 100 of these carbohydrate antigens have been elucidated. Most of them are related to the human ABO and Lewis blood group antigens. The antibodies are being used to study changes in cell surface carbohydrates during development in hopes of providing insights into the functions of glycoconjugates. For example, antibody LeoMel 3 which is directed against the ganglioside GD3, specifically inhibits killing of melanoma cells by human anomalous killer cells. These killer cells differentiate from classical cytotoxic T lymphocytes and lyse freshly isolated human melanoma cells which are insensitive to natural killer cell-mediated lysis. It is likely that the killer cells recognize the sugar sequence of ganglioside GD3. Antibody LeoMel 3 is an IgM. Other IgM antibodies from the same fusion that bind melanoma cells do not inhibit killer cell-mediated lysis. Another antibody, 18B8, which is directed against the ganglioside GT3, reacts only with the inner and outer synaptic layers of developing chick retina. It is possible that the sugar sequence of ganglioside GT3 is involved in synapse formation possibly by binding to lectin-like molecules on neuronal cell surfaces.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 57001-09 LSB
Formerly
Z01 AM 18004-09 LBM

PERIOD COVERED
October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Metabolism and Role of Polysaccharide Sulfates

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Irwin G. Leder	Research Chemist	LSB	NIDDK

COOPERATING UNITS *(if any)*
Allen Minton, LBP, NIDDK
William Poillon, Center for Sickle Cell Disease, Howard University

LAB/BRANCH
Laboratory of Structural Biology

SECTION
Section on Biochemistry

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The discovery of a novel sulfatase of unusual specificity and the synthesis of isomeric glucosamine sulfates of known structure have led to the discovery that heparin contains a 3-O sulfated glucosamine residue which is essential for its role as an anticoagulant. The enzyme, which has been partially purified from pooled human urine is present as several differently charged forms.

Many polyanions, including heparin, induce allosteric changes in the structure of hemoglobin. In a study of the effects on hemoglobin of polyanions of controlled size, highly sulfated trehalose and stachyose have been prepared. These compounds have been found to bind with high affinity to hemoglobin-S and strongly decrease its affinity for oxygen. Studies of the effects of these and related compounds on the solubility of hemoglobin-S are being carried out.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 57002-12 LSB
Formerly
Z01 AM 17006-12 LBM

PERIOD COVERED
October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Expression and Function of Bacterial Cell Surface Components

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	John Foulds, Ph.D.	Research Biochemist	LSB	NIDDK
Others:	Victor Jiminez, MD, M.Sc.	Staff Fellow	LSB	NIDDK
	Gretchen Schieber, Ph.D.	Staff Fellow	LSB	NIDDK
	Susan Stickley, DDS	Guest Researcher	LSB	NIDDK

COOPERATING UNITS *(if any)*
Keith Joiner and Richard Silver, LCI, NIAID

LAB/BRANCH
Laboratory of Structural Biology

SECTION
Section on Membrane Biology

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	4.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The E. coli K1 capsule is involved in pathogenic processes such as virulence and invasiveness. We find that expression of K1 capsule synthesis on the bacterial cell surface requires the presence of porin proteins in the outer membrane. A specific porin, protein K, found in all K1 encapsulated strains, is able to support the more rapid expression of K1 capsule as compared to other porin proteins tested. There was, however, no increase in the steady state amount of K1 capsule in strains containing protein K. Thus, protein K may be more efficient in the organization of a putative outer membrane complex involved in the extracellular transport on the K1 capsule.

Bacteria are ingested and degraded by macrophages. However, certain bacterial components such as LPS may persist for several days within the macrophage after phagacytosis. Outer membrane proteins also persist. It is notable that these proteins are associated with LPS within the cell and their persistance may well be a reflection of this association. The bacterial components resistant to degradation may subsequently be cycled to the cell surface for antigen presentation.

The O-PS portion of LPS in the outer membrane of Salmonellae regulates the extent of deposition of the complement component C3b and hence regulates the rate of phagacytosis of these cells by macrophages. Relatively minor changes in the structure of different O-PS found in various strains may have profound effects on the clearance of these cells and thus alter their virulence in mice. The O-PS functions in this process by influencing the activation of complement by the alternate pathway by altering the binding of factor B. and hence the amplification of deposition of C3b.

ANNUAL REPORT
THE LABORATORY OF MOLECULAR AND CELLULAR BIOLOGY

NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

The LMCB was established in May 1985 with Barrie J. Carter (formerly Chief, Section on Macromolecular Genetics, LCBG) as Chief. This is the first formal annual report of LMCB. The LMCB now comprises two major groups. One group, led by Barrie J. Carter, studies gene regulation in mammalian cell systems and is particularly interested in developing efficient vector systems for delivery of genes into cells. The second group, led by Takami Oka, is generally interested in the endocrine control of differentiation of the mouse mammary gland and has focused on physiological effects of EGF and the molecular biology of various genes which are important in this process.

During the past year, as a result of the recent moves of component parts of the laboratory from Building 4 and Building 10 to Building 8, the personnel of LMCB have become geographically located on the same floor. This is expected to greatly improve interaction and collaboration between different groups. Both of the major groups in this laboratory have continued very active and productive research programs and work has been presented at a series of national and international meetings.

Function of DNA Virus Genomes in Animal Cells

The group led by B. Carter has continued to employ DNA viruses as molecular probes to study genome expression in human cells. We are studying intensively the structure and function of adeno-associated virus (AAV) since this virus has only a small genome. AAV has also been developed as a eukaryotic expression vector. AAV normally grows in cells only in the presence of a helper virus (either adenovirus or herpesvirus). In the absence of any helper the AAV genome integrates into the cell chromosome. Thus, the AAV vector is useful as a transducing virus for high frequency integration of genes in mammalian cell chromosomes to yield stable expression. This vector also may be useful for therapy. Award of a patent for this vector system is imminent. We are now analyzing intensively the control of gene regulation in AAV vectors in order to maximize the expression of foreign genes introduced into mammalian cells using this vector. We have discovered a complex system of gene regulation mediated by products of the AAV rep gene which are required for replication of AAV DNA but also mediate transcriptional activation and also translational inhibition of some genes. Coding of all these functions in a single gene appears to be unique in eukaryotic systems. We are also studying adenovirus since this is the helper virus for AAV multiplication. This helper relationship is being analyzed. Also, both AAV and adenovirus recombine with cellular DNA. In the case of adenovirus, this causes malignant transformation of the cell. AAV inhibits this transformation and also inhibits AD12 oncogenesis in newborn animals. The mechanism of this inhibition of tumor induction by AAV is being studied at the molecular level in both cell culture and in

animal experiments. We are also studying mutations in mouse 3T3 cells which render the cells resistant to malignant transformation by a single oncogene (ras) but allow malignant transformation by two oncogenes (ras, myc) acting in concert.

Hormonal Regulation of Cell Growth and Differentiation

The group led by T. Oka has continued studies on epidermal growth factor (EGF), a polypeptide consisting of 53 amino acid residues that is produced by the mouse submandibular gland. EGF has diverse biological actions influencing proliferation, differentiation, and functional activities of various types of cells. However, the physiological role of EGF has not been well-defined. Our previous studies indicate that submandibular gland-derived EGF plays a role in the development of the mammary gland during pregnancy and mammary tumorigenesis in female mice. By contrast, the physiological role of EGF in males is not clear, despite knowledge that the concentration of EGF in the submandibular gland and plasma of male mice is much higher than that of females. In males, the production of EGF increases in parallel with sexual maturation, and androgens stimulate its production in the submandibular gland. We have examined the effect of sialoadenectomy and EGF replacement on several parameters of male reproductive function, including sperm production. Our results indicate that sialoadenectomy decreases the amount of circulating EGF to an undetectable level, but does not affect the circulating levels of testosterone and FSH. The number of mature sperm in the epididymis decreases by about 55%; the number of spermatids in the testis decreases 40-50%; and the number of spermatocytes increases about 20%. Administration of EGF to sialoadenectomized mice completely corrected both the sperm content of the epididymis and the number of spermatids in the testis. Thus, EGF may serve a role in male reproduction by stimulating the meiotic phase of spermatogenesis through a submandibular gland-testis axis.

The most important question in the study of actions of polypeptide hormones deals with identifying the steps in the transduction chain. We examined the effect of EGF on the membrane potential and ion channels of mammary epithelial cells by electrophysiological techniques. These studies reveal that EGF induces a hyperpolarizing response by stimulating the Ca dependent K^+ channel. This response may be closely related to the mechanisms responsible for the mitogenic action of EGF.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 57501-10 LMCB (formerly Z01 AM 21010-09 LCBG)

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Function of DNA Virus Genomes in Animal Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Barrie J. Carter	Chief, Laboratory of Molecular and Cellular Biology	LMCB:NIDDK
Others:	Jacov Tal	Visiting Scientist	LMCB:NIDDK
	Ella Mendelson	Visiting Associate	LMCB:NIDDK
	Christeine Lally	Visiting Fellow	LMCB:NIDDK
	James Trempe	Guest Worker	LMCB:NIDDK
	Nor Chejanovsky	Visiting Fellow	LMCB:NIDDK
	Irving Miller	Biologist	LMCB:NIDDK

COOPERATING UNITS *(if any)*

V. Nikodem, S. Usala CEB, NIDDK; B. Weintraub, F. Wondisford, MCEB, NIDDK M.G. Smith, Univ. Otago, New Zealand; J. Tratschin, Univ. Bern, Switzerland, E. Katz, Hebrew Univ., Jerusalem.

LAB/BRANCH

Laboratory of Molecular and Cellular Biology

SECTION

INSTITUTE AND LOCATION

NIDDK:NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8.0	7.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are employing DNA viruses as molecular probes to study genome expression in human cells. We are studying intensively the structure and function of adeno-associated virus (AAV) since this virus has only a small genome. AAV has also been developed as a eukaryotic expression vector. AAV normally grows in cells only in the presence of a helper virus (either adenovirus or herpesvirus). In the absence of any helper, the AAV genome integrates into the cell chromosome. Thus, the AAV vector is useful as a transducing virus for high frequency integration of genes into mammalian cell chromosomes to yield stable expression. This vector also may be useful for gene therapy. We are now analyzing intensely the control of gene regulation in AAV vectors in order to maximize the expression of foreign genes introduced into mammalian cells using this vector. We have discovered a complex system of gene regulation mediated by products of the AAV rep gene which are required for replication of AAV DNA but also mediate transcriptional activation and also translational inhibition of some genes. Coding of all these function in a single gene appears to be unique in eukaryotic systems. We are studying also adenovirus which is a more complex genome. Adenovirus is the helper virus for AAV multiplication. This helper relationship is being analyzed. Also, both AAV and adenovirus recombine with cellular DNA. In the case of adenovirus this causes malignant transformation of the cell. AAV inhibits this transformation and also inhibits Ad12 oncogenesis in newborn animals. Thus, AAV inhibits tumor induction. The mechanism of this inhibition of tumor induction is being studied at the molecular level in both cell culture and in animal experiments. We are also studying mutations in mouse 3T3 cells which render the cells resistant to malignant transformation by a single oncogene (ras) but allow malignant transformation by two oncogenes (ras, myc) acting in concert.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 57502-13 LMCB
(formerly
Z01 AM 18003-12 LBM)

PERIOD COVERED
October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Hormonal Regulation of Cell Growth and Differentiation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Oka, T., Senior Investigator, LMCB, NIDDK

Others:	Borellini, F.	Visiting Fellow	LMCB, NIDDK
	Perry, J.W.	Biologist	LMCB, NIDDK
	Tsutsumi, A.	Guest Worker	LMCB, NIDDK
	Tsutsumi, O.	Visiting Fellow	LMCB, NIDDK
	Yoshimura, M.	Visiting Fellow	LMCB, NIDDK
	Vijay, I.K.	Guest Worker	LMCB, NIDDK

COOPERATING UNITS *(if any)*
Dr. Charles Edwards, LCBG, NIDDK
Dr. G. Mezzetti, University of Modena, Italy

LAB/BRANCH
Laboratory of Molecular and Cellular Biology

SECTION

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.0	5.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use stenderd unreduced type. Do not exceed the spece provided.)*

Epidermal growth factor (EGF), a polypeptide consisting of 53 amino acid residues, is produced by the mouse submandibular gland. EGF has diverse biological actions influencing proliferation, differentiation, and functional activities of various types of cells. However, the physiological role of EGF has not been well-defined. Our previous studies indicate that submandibular gland-derived EGF plays a role in the development of the mammary gland during pregnancy and mammary tumorigenesis in female mice. By contrast, the physiological role of EGF in males is not clear, despite knowledge that the concentration of EGF in the submandibular gland and plasma of male mice is much higher than that of females. In males, the production of EGF increases in parallel with sexual maturation, and androgens stimulate its production in the submandibular gland. We have examined the effect of sialoadenectomy and EGF replacement on several parameters of male reproductive function, including sperm production. Our results indicate that sialoadenectomy decreases the amount of circulating EGF to an undetectable level, but does not affect the circulating levels of testosterone and FSH. The number of mature sperm in the epididymis decreases by about 55%; the number of spermatids in the testis decreases 40-50%; and the number of spermatocytes increases about 20%. Administration of EGF to sialoadenectomized mice completely corrected both the sperm content of the epididymis and the number of spermatids in the testis. Thus, EGF may serve a role in male reproduction by stimulating the meiotic phase of spermatogenesis through a submandibular glandtestis axis.

The most important question in the study of actions of polypeptide hormones deals with identifying the steps in the transduction chain. We examined the effect of EGF on the membrane potential and ion channels of mammary epithelial cells by electrophysiological techniques. These studies reveal that EGF induces a hyperpolarizing response by stimulating the Ca^{2}-dependent K^{+} channel. This response may be closely related to the mechanisms responsible for the mitogenic action of EGF.

ANNUAL REPORT OF THE LABORATORY OF ANALYTICAL CHEMISTRY

NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

SECTION ON INSTRUMENTATION

SERVICE FUNCTIONS AND INSTRUMENTATION

Basic research and service functions are performed by members of the Section. A major mission of the organization involves the instrumental and chemical analyses provided to scientist of the Laboratory of Chemistry, Laboratory of Bioorganic Chemistry, NIH and to a limited extent to personnel of other government agencies. Instrumental analyses include: GC/MS spectrometry, gas-liquid chromatography, infrared, nuclear magnet resonance, ultraviolet and flame photometry. Assistance in interpretation of spectra is rendered on request. Samples for microanalysis are handled by external contracts. (D.F. Johnson, H.J.C. Yeh, N. Whittaker, W. White).

APPLICATIONS OF NMR IN BIOCHEMICAL AND BIOLOGICAL SYSTEMS:

The objective of this project is to develop and apply nuclear magnetic resonance for elucidating molecular structures and for studying the interactions within and between molecules in making contribution to the solution of various chemical problems.

Various nmr techniques have been employed 1) to determine absolute stereochemistries of the 5,6-oxide formed from 7,12-dimethylbenz[a]-anthracene by cytochrome P450c and benzo[c] phenanthrene 5,6-oxide and other K-region derivatives; 2) to elucidate the structures of eight principal adducts formed from the deoxyguanosine residues of DNA upon reaction in vitro with four configurationally isomeric 3,4-diol-1,2-epoxides; and 3) to demonstrate that tryptophan synthase can racemize the carbon of 5-fluoro-L-tryptophan and its 2,3-dihydro derivatives.

Metabolites of polycyclic aromatic hydrocarbons(PAH) formed by liver microsomal cytochromes P450 and epoxide hydrolase are frequently found in high optical yields. Over the years, optical purity and absolute stereochemistry of several classes of bay-region diol epoxides and K-region oxides prepared for PAH have been established by a variety of nmr techniques, e.g. 2D-nmr and nmr in the presence of chiral lanthanide shift reagents. These results have provided valuable information toward the understanding of the mechanism of action of these enzymes. As we are also interested in the ultimate role of the PAH-epoxides in tumorigenicity, studies on the nucleophilic opening of the epoxides have special significance. We have, thus, conducted an nmr investigation on reaction of 7,12 dimethylbenz[a] anthracene 5,6-oxide with various nitrogen and oxygen nucleophiles. The results of this study allows us to establish the regioselectivity of the nucleophilic attack on DMBA 5,6-oxide. We now extend these studies to PAH-oxide adducts with DNA. The stereochemistry of eight principal adducts of DNA upon reaction in vitro with four configurationally isomeric 3,4-diol-1,2-epoxides were assigned on the basis of their ^{1}H nmr spectra. This results revealed that the site of covalent attachment of the diol epoxide moiety to the nucleoside residue in these adducts is at the

exocyclic amino group and this nitrogen is linked to C_1 of the tetrahydrobenzo(c) phenanthrene system.

Fluorine-19 nuclear magnetic resonance has been used to study the binding and reactions of the D and L isomers of 5-fluorotryptophan, of tryptophan, and of (3S)-and (3R)-2,3-dihydro-5-fluorotryptophan. tryptophan synthase specifically and tightly binds the (3S) diastereoisomer of both 2,3-dihydro-5-fluoro-D-tryptophan and 2,3-dihydro-5-fluoro-L-tryptophan, whereas it binds 5-fluoro-D-tryptophan more tightly than 5-fluoro-L-tryptophan. Unexpectedly, we find that the D and L isomers of 5-fluorotryptophan and of (3S)-2,3-dihydro-5-fluorotryptophan are slowly interconverted by isomerization reactions. Since these isomerization reactions are 10^3-10^5 times slower than the -replacement and -elimination reactions catalyzed by tryptophan synthase, they have no biochemical significance in vivo. However, the occurrence of these slow reactions does throw some light on the nature of the site of tryptophan synthase and its requirements for substrate binding. (H.J.C. Yeh, D.M. Jerina, J.M. Sayer, S.K. Balani, E.W. Miles, L.A. Cohen, R.S. Phillips and H. Yagi).

SECTION ON STEROID HORMONES

NATURE OF STEROID-RECEPTOR INTERACTIONS

The object of this project is to define the initial, intracellular events of steroid hormone action. These events include steroid binding to the intracellular receptor molecule, "activation" of the receptor-steroid complex to a DNA-binding and nuclear-binding species, and binding of the activated complex to those nuclear acceptor sites involved in the regulation of transcription of specific genes. One approach that has been used to examine these steps is to compare the properties of agonist and antagonist receptor-steroid complexes. Detailed studies of the amount of induction of tyrosine aminotransferase (TAT) by several antiglucocorticoids in two rat hepatoma tissue culture lines (HTC and Fu5-5) revealed that each antiglucocorticoid displayed about 2-fold more agonist activity in Fu5-5 cells than in HTC cells. At the same time, it was noted that the concentration of several glucocorticoids required for 50% of maximal TAT induction (i.e., EC_{50}) in Fu5-5 cells were about 6-fold lower than in HTC cells. The values of both parameters varied over time. A retrospective analysis revealed an excellent linear, reciprocal relationship between the amount of agonist activity for the irreversible antiglucocorticoid dexamethasone 21-mesylate and the EC_{50} of the glucocorticoid dexamethasone ($R=-0.896[n=46]$). These changes in TAT enzyme activity were paralleled by changes in the amount of TAT mRNA sequences but were not observed with another glucocorticoid inducible enzyme, glutamine synthetase. Thus we have documented a novel modulation of glucocorticoid induction of TAT transcripts. Such modulation has not been observed for the control of gene transcripts by any other steroid hormones but may be related to the known variation in agonist activity seen for most antisteroids in vivo in different systems. (S.S. Simons, Jr., P.A. Miller, P. Yen, G. Wasner, F. Sistare, A. Cavanaugh and N. Miller).

THE DEVELOPMENT OF METHODS AND MATERIALS FOR THE STUDY OF MEDICAL PROBLEMS

The biology, chemistry, and genetics of cancer metastasis are being studied to increase our understanding of cell biology in general and to provide leads for the development of new agents for the treatment of cancer and metastases. The immediate objective is to determine whether specific gene products are required for the formation of metastases from cells of the primary tumor or lung colonies from circulating tumor cells. Cell lines and sublines have been developed from transfections of the immortal but non-tumorigenic NIH 3T3 cells with constructs of src, v-abl, c-mos, and v-mos oncogenes. Cell lines containing the src and v-mos oncogenes have been injected subcutaneously and intravenously into nude mice. In all cases the cells were tumorigenic and non-metastatic and produced no or relatively few lung colonies. The correlation between expression of the introduced oncogenes and tumorigenic, metastatic, and lung colonizing potencies is being determined. These cells lines are good candidates for attempts to increase metastatic and lung colonizing potencies by further transfections. Success in these attempts might permit the determination of specific genetic requirements for metastasis. (C.M. Foltz, R. Muschel, and L.A. Liotta).

SECTION ON BIOPHYSICAL HISTOLOGY

A Rhodamine for Intracellular Injection

Work was in progress to synthesize a rhodamine that can be used, in conjunction with Lucifer Yellow CH, for intracellular injection. The compound, a tetrasulfonated dihydrazidorhodamine, has been synthesized and shows promise for intracellular injection.

Genetics of Nerve Cell Shape

Studies have continued on the genetics of nerve cell shape, using as an experimental animal the snail Biomphalaria glabrata. B. glabrata was chosen because of its suitability for neurophysiology, neuroanatomy, genetics, and convenient rearing in the laboratory.

Professional Practices of a Group of Biomedical Scientists

Studies are in progress on the professional practices of a group of biomedical scientists. This is a group about whose practices there is an unusual amount of information available in published documents. A report on this study is still undergoing revision. We had been advised to stop work on the project on August 1, but, have been given an extension (N. Feder and Walter Stewart).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 58000-41LAC

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Service Functions and Instrumentation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Dr. David F. Johnson	Chief, Lab. Anal. Chem	NIDDK/LAC
OTHERS:	Dr. Herman J. C. Yeh	Research Chemist	NIDDK/LAC
	Mr. Noel Whittaker	Chemist	NIDDK/LAC
	Mr. Westly White	Biologist	NIDDK/LAC

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Analytical Chemistry

SECTION

Instrumentation Section

INSTITUTE AND LOCATION

NIH, NIADDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	4.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Basic research and service functions are performed by members of the Section. A major mission of the organization involves the instrumental and chemical analyses provided to scientist of the Laboratory of Chemistry, Laboratory of Bioorganic Chemistry, NIH and to a limited extent to personnel of other government agencies. Instrumental analyses include: GC/MS spectrometry, gas-liquid chromatography, infrared, nuclear magnet resonance, ultraviolet and flame photometry. Assistance in interpretation of spectra is rendered on request. Samples for microanalysis are handled by external contracts.

Formerly Z01 AM 19801-40 LC

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 58001-13LAC

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 cherecters or less. Title must fit on one line between the borders.)*
Applications of NMR in Biochemical and Biological Systems

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principel Investigetor.) (Name, title, laboratory, and institute affiliation)*

P.I.	Dr. Herman Yeh	Research Chemist	NIDDK/LAC
Others:	D.M. Jerina	Sec. Chief	NIDDK/LBC
	A. Brossi	Sec. Chief	NIDDK/LC
	P. Kovac	Visiting Assoc.	NIDDK/LC
	C.P. Glaudemans	Sect. Chief	NIDDK/LC
	J.M. Sayer	Research Chemist	NIDDK/LBC
	S.K. Balani	Visiting Fellow	NIDDK/LBC

COOPERATING UNITS *(if any)*
D.E. Ryan, P.E. Thomas, A.H. Conney and W. Levin (Roche Inst. of Molecular Biology). D.R. Thakker (Bureau of Biologic)

LAB/BRANCH
Laboratory of Analytical Chemistry

SECTION
Instrumentation

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	05.	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the spece provided.)*

The objective of this project is to develop and apply nuclear magnetic resonance for elucidating molecular structures and for studying the interactions within and between molecules in making contribution to the solution of various chemical problems.

Various nmr techniques have been employed 1) to determine absolute stereochemistries of the 5,6-oxide formed from 7,12-dimethylbenz[a]-anthracene by cytochrome P450c and benzo[c] phenanthrene 5,6-oxide and other K-region derivatives; 2) to elucidate the structures of eight principal adducts formed from the deoxyguanosine residues of DNA upon reation in vitro with four configurationally isomeric benzo[c]phenanthrene 3,4-diol-1,2-epoxides; and 3) to demonstrate that tryptophan synthase can racemize the carbon of 5-fluoro-L-tryptophan and its 2,3-dihydro derivatives.

Formerly Z01 AM 19803-12 LC

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 58002-11LAC

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Nature of Steroid-Receptor Interactions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	S. Stoney Simons, Jr.,	Chief, Steroid Hormones Section	NIDDK/LAC
OTHERS:	Patricia A. Miller	Staff Fellow	NIDDK/LAC
	Gertraud Wasner	Visiting Fellow	NIDDK/LAC
	Frank D. Sistare	PRAT Fellow	NIDDK/LAC
	Paul M. Yen	Intramural NRSA Fellow	NIDDK/LAC
	Alice Cavanaugh	Extramural NRSA Fellow	NIDDK/LAC
	Nancy R. Miller	Special Expert	NIDDK/LAC

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Analytical Chemistry

SECTION
Steroid Hormones

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.0	6.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The object of this project is to define the initial, intracellular events of steroid hormone action. These events include steroid binding to the intracellular receptor molecule, "activation" of the receptor-steroid complex to a DNA-binding and nuclear-binding species, and binding of the activated complex to those nuclear acceptor sites involved in the regulation of transcription of specific genes. One approach that has been used to examine these steps is to compare the properties of agonist and antagonist receptor-steroid complexes. Detailed studies of the amount of induction of tyrosine aminotransferase (TAT) by several antiglucocorticoids in two rat hepatoma tissue culture lines (HTC and Fu5-5) revealed that each antiglucocorticoid displayed about 2-fold more agonist activity in Fu5-5 cells than in HTC cells. At the same time, it was noted that the concentration of several glucocorticoids required for 50% of maximal TAT induction (i.e., EC_{50}) in Fu5-5 cells were about 6-fold lower than in HTC cells. The values of both parameters varied over time. A retrospective analysis revealed an excellent linear, reciprocal relationship between the amount of agonist activity for the irreversible antiglucocorticoid dexamethasone 21-mesylate and the EC_{50} of the glucocorticoid dexamethasone (R=-0.896[n=46]). These changes in TAT enzyme activity were paralleled by changes in the amount of TAT mRNA sequences but were not observed with another glucocorticoid inducible enzyme, glutamine synthetase. Thus we have documented a novel modulation of glucocorticoid induction of TAT transcripts. Such modulation has not been observed for the control of gene transcripts by any other steroid hormones but may be related to the known variation in agonist activity seen for most antisteroids in vivo in different systems.

Formerly Z01 AM 19806-11 LC

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 58003-13 LAC

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Development of Methods and Materials for the Study of Medical Problems.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Calvin M. Foltz Research Chemist

OTHERS: Byron Baer Chemist

COOPERATING UNITS *(if any)*

Lance A. Liotta and Ruth Muschel, Pathologists, Laboratory of Pathology, NCI

LAB/BRANCH

Laboratory of Analytical Chemistry

SECTION

Steroid Hormones

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The primary goal of this work is to contribute to the investigation and solution of basic medical problems by the application of chemical, physical and biological methods. This goal is being pursued by studies of the biology and molecular biology of murine tumor cells with emphasis on cancer metastasis. Areas of special interest are organic chemistry, biochemistry, cell biology, tissue culture, cancer biology, cancer chemotherapy and recombinant DNA methodology.

Studies are being conducted to determine whether specific gene products confer on certain tumor cells the properties required for the formation of viable metastases. NIH 3T3 cells have been transfected with constructs of several oncogenes. Transformed cells have been selected and their tumorigenic and metastatic potencies determined by subcutaneous and tail vein injections in nude mice. The correlation of turmorigenic and metastatic potencies with the expression of the oncogene introduced is being determined.

Additional transfections of certain cell lines, e.g., those with tumorigenic but not spontaneous metastatic potency and with or without lung colonizing potency will be performed in an attempt to endow the cells with the properties necessary for spontaneous metastasis. Success in this would increase our knowledge of the genetic requirements for metastasis.

Formerly Z01 AM 19804-12 LC

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 58004-19LAC

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Histochemistry: Principles, Methods and Applications

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	N. Feder	Medical Officer (Research)	NIDDK/LAC
Others:	W. Stewart	Research Physicist	NIDDK/LAC

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Analytical Chemistry

SECTION
Section on Biophysical Chemistry

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	2	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Continuing studies with the dye, Lucifer Yellow, have been carried out.

Studies have continued on the genetics of nerve cell shape using the snail Biomphalaria glabrata as an experimental animal.

Studies are continuing on the professional practices of a group of biomedical scientists.

Formerly Z01 AM 21005-18 LCBG

PHS 6040 (Rev. 1/84) GPO 914-918

ANNUAL REPORT OF THE PHOENIX EPIDEMIOLOGY AND CLINICAL RESEARCH BRANCH,

National Institute of Diabetes and Digestive and Kidney Diseases

Overview:

The Phoenix Epidemiology and Clinical Research Branch is an integral part of the Intramural Research Program of the Institute. The Branch is located in Phoenix, Arizona and performs research in diabetes, digestive and kidney diseases, with emphasis on conditions which are particularly prevalent among the American Indians of the Southwest. The Branch works in close collaboration with the Indian Health Service and the Gila River Indian Community. The Branch is responsible for investigation of the epidemiology as well as clinical and laboratory research pertaining to these diseases. Of particular importance to the activities of the Branch is the unique Facility to study these diseases in a well-defined population. Through the conduct of field studies, and then, based on the characteristics of the population the Branch is able to design studies and recruit volunteers for specific clinical and laboratory research based on the individual and family characteristics. Furthermore, because of the well-defined and closed nature of the Indian communities, particularly the Pima Indians of the Gila River Indian Community long-term followup studies and reinvestigation of the same individuals many years later is possible.

Population based studies performed by the Diabetes and Arthritis Epidemiology Section have shown that the Pima Indians have the highest prevalence and incidence of diabetes in the world, which is accompanied by all of the classical, vascular complications, including diabetic retinopathy and nephropathy, as well as complications of pregnancy, and an extraordinarily high frequency of cholelithiasis. The epidemiologic investigations provides a basis for studies of the determinants of diabetes itself, as well as risk factors and natural history of the complications. In addition, the population has been the basis for the research on diabetes and obesity carried out by the Clinical Diabetes and Nutrition Section, which includes clinical research studies of the mechanisms and pathogenesis of diabetes mellitus, obesity and lipoprotein metabolism. The Branch also contains a Biostatistics and Data Management Section whose responsibilities are to supply biostatistical and data management support for the other sections.

Other Professional Activities

The staff of the Branch continue to be active in international and national organizations. Dr. William Knowler was elected Chairman of the Epidemiology and Health Statistics Council of the American Diabetes Association and Dr. Clifton Bogardus was elected to membership in the American Society of Clinical Investigation. Several members of the Branch serve on editorial boards of major scientific journals and serve as advisors and consultants for other major activities. In particular, several of the staff have assisted the National Center for Health Statistics in the planning of the proposed National Health And Nutrition Examination Survey (NHANES) III, and with analysis and publication of data from NHANES II relating to diabetes. Two members have participated as invited speakers in meetings of the National Research Council of the National Academy of Sciences and Dr. Bennett, the Branch Chief, delivered the first American Diabetes Association Kelly West Memorial Lecture.

International Activities

Members of the Branch continue to plan an important role in international activities. The Diabetes and Arthritis Epidemiology Section is a participant in the WHO Multinational Study of Vascular Disease in Diabetes. Dr. Barbara Howard, Associate Chief of the Clinical Diabetes and Nutrition Section has been engaged in collaborative research in Finland and in Milan, Italy, and Dr. William Knowler, Chief of the Diabetes and Arthritis Epidemiology Section, has collaborated with investigators from the University of Lund, Sweden in the analysis of the data relating to impaired glucose tolerance, and the design of new studies in this field. In addition, several members of the staff have been invited international and national conferences to deliver invited lectures.

During past year the Branch has been selected by the World Health Organization (WHO) and designated as the WHO Colllaborating Centre for the Design and Methodology and Analysis of Epidemiology and Clinical Research in Diabetes.

DIABETES AND ARTHRITIS EPIDEMIOLOGY SECTION

The Diabetes and Arthritis Epidemiology Section has continued its 21-year longitudinal study of genetic and environmental risk factors for diabetes and vascular complications of diabetes in the Pima Indians, as well as data collection for epidemiological studies of rheumatoid arthritis, ankylosing spondylitis, osteoarthritis, cholelithiasis, mortality rates and causes of death in the Gila River Indian Community.

The natural history of impaired glucose tolerance (IGT) has been investigated. Subjects with this condition, while manifesting none of the symptoms or complications of diabetes, are at high risk of developing diabetes in the future. Among subjects with IGT diagnosed for the first time, the rate of development of diabetes was influenced by age, obesity, and fasting and post-load serum insulin concentrations in a manner suggesting that insulin resistance leads to IGT, and inadequate insulin secretion in response to a challenge results in further decompensation to diabetes.

Diabetes complications are being documented and their risk factors determined. Major complications of diabetes under study are nephropathy, end stage renal disease, retinopathy, peripheral vascular disease, cataracts, and periodontal disease, all of which appear to develop as least as frequently in this population with non-insulin dependent diabetes as reported in other studies insulin dependent diabetes.

The adverse affects of diabetes in pregnancy, both for the mother and for the offspring, are being evaluated in the Pima Indians. Offspring of diabetic women, even if they escape morbidity and are not overweight in the newborn period, are at an increased risk of obesity, glucose intolerance and diabetes during childhood and young adulthood. The causes of these adverse outcomes is being investigated by including measurement of proinsulin, C-peptide, and glycosylated hemoglobin in cord blood and glycosylated hemoglobin in maternal blood. The diabetes status of the fathers of these offspring is also determined so that familial and genetic factors can be evaluated as well as the effects of the intrauterine environment.

Collection and analysis of data on cause of death on all deceased subjects and of tissues from autopsied subjects continues, although the collection of autopsy data has been severely hampered by the declining autopsy rate.

The Section staff continue to be active in medical research and education beyond the projects described here. Section staff collaborate extensively in research projects conducted at the Clinical Diabetes and Nutrition Section of NIDDK and the National Center of Health Statistics, contribute to several national and international meetings and workshops, and are developing plans for a multicentered international clinical trial in impaired glucose tolerance.

CLINICAL DIABETES AND NUTRITION SECTION

Research at the Clinical Diabetes and Nutrition Section is in three major areas: non-insulin dependent diabetes mellitus; obesity and energy balance; and, lipoprotein metabolism.

Non-Insulin Dependent Diabetes Mellitus

The Pima Indians of the Gila River Indian Community have the highest reported prevalence and incidence of non-insulin dependent diabetes mellitus (NIDDM) of any population in the world. Diabetes occurs more often in the offspring of diabetic mothers than in the offspring of nondiabetic parents. The major effort of the Clinical Diabetes and Nutrition Section continues to be a longitudinal study of the offspring of these two parental types. Obese offspring of diabetic mothers and of nondiabetic parents are admitted yearly to the clinical unit for detailed studies of many aspects of carbohydrate metabolism, including both in vivo and in vitro studies. Based on previously collected epidemiologic data, approximately 30% of the obese offspring of diabetic mothers will develop NIDDM within five years such that it will be possible to carefully document the sequence of metabolic events that occurs as subjects with normal glucose tolerance develop diabetes. This will enable determination of which metabolic parameter, specifically an abnormality of insulin secretion or of insulin action, is predictive of the development of NIDDM.

Approximately 250 subjects have been entered into this study, 150 subjects have returned for their second visit, about 60 subjects have returned for their third visit, and about 40 subjects have now returned for a fourth examination. We are now beginning to admit subjects for their fifth year in the study. From initial cross-sectional analyses some surprising and important observations have emerged. Degree of obesity accounts for only approximately 50% of the variance of insulin action observed in the population. In fact, obesity is not linearly related to the degree of insulin resistance at low plasma insulin concentrations. Maximal oxygen uptake, as an estimate of physical fitness, is linearly related to insulin action at all measured insulin concentrations, but accounts for only about 25% of the variance of observed insulin action. Since most of the variance in insulin action is not explained by degree of obesity and degree of physical fitness, as perhaps generally thought, we have explored other possible determinants of insulin action in vivo in man.

A study of the relationship between skeletal muscle morphology and insulin action has recently been completed. This has shown that capillary density is significantly correlated with degree of insulin action in vivo. In addition, a relationship between the percent of type IIB muscle fibers (glycolytic fibers) and insulin action has been found. This parallels work by other groups in animals which have demonstrated that the type I fibers are insulin sensitive and that type II fibers are insulin resistant. It may well be that the percent type II fiber is an inherited characteristic associated with a predisposition to insulin resistance.

A familial analysis of insulin resistance has shown that degree of insulin resistance is a strongly familial characteristic in this population, even after adjusting for familial differences in degree of obesity and physical fitness. These insulin resistance and insulin sensitive families will form the nucleus for future genetic studies.

Indirect calorimetric data collected during the performance of euglycemic clamps, as a measure of insulin action in vivo, has demonstrated that non-oxidative pathways are the major pathway for glucose disposal during insulin infusion. In vitro studies done to determine the possible mechanisms of non-oxidative insulin mediated glucose disposal have shown that the rate of insulin mediated glucose storage is well correlated with insulin activation of the human skeletal muscle enzyme glycogen synthase. Experiments have also been performed to determine if the ED_{50} for insulin activation of non-oxidative disposal and total insulin mediated glucose disposal in vivo is parallel to the ED_{50} for insulin activation for human skeletal muscle glycogen synthase and have shown that the ED_{50}s for these processes are all similar. These data suggest that glycogen synthase may even be a rate limiting step for insulin action in vivo in insulin resistant man. To explore this further, substrate concentrations of glucose-6-phosphate in insulin sensitive and insulin resistant subjects before and after insulin stimulation have been determined. The glucose-6-phosphate concentration in the skeletal muscle was shown to be higher in insulin resistant subjects than in insulin sensitive subjects, suggesting that there may be a block in insulin mediated glucose metabolism in insulin resistant subjects after glucose-6-phosphate. Thus, there may be a rate limiting step, such as muscle glycogen synthase, for insulin action in insulin resistant subjects in addition to the previously hypothesized abnormality of glucose transport.

The mechanisms of control oxidative glucose disposal in response to insulin infusion in man have been explored further. The rate of oxidative glucose disposal was previously demonstrated to be related to the free fatty acid concentration in plasma, as previously suggested by Randall. These experiments have been expanded to determine if there was a relationship between the rate of free fatty acid release and oxidative disposal as well as whether there was a relationship between the lipolytic rate in vitro in isolated adipocytes and the rate of free fatty acid release and, thereby also the rate of oxidative disposal. The rate of free fatty acid release was found to be not well correlated with the size of the lipid mass of the body, as if obese subjects have fat in their adipose tissue which is not readily releasable to the general circulation. There was also little relationship between the free fatty acid turnover rate as measured by labelled palmitate infusion and the rate of lipid oxidation and glucose utilization. Thus, there was a significant portion of the free fatty acid production that was non-oxidized. The fate of non-oxidized free fatty acid clearance remains unknown.

Obesity and Energy Balance

It has been proposed that the high prevalence of obesity among the Pima Indians may be due to genetic selections for a "thrifty" gene. To determine if differences in metabolic rate exist between Indians and Caucasians, or among individuals within the Indian population, we have measured rates of energy expenditure in the resting condition using indirect calorimetry. In addition, a human respiratory chamber has been constructed which allows measurement of rates of energy expenditure over 24-hour periods. In addition, the chamber can be used to measure substrate utilization rates throughout the day in response to meals and sleep.

A Very strong familial aggregation of resting metabolic rate has been found which is independent of family differences in metabolic size, age, and sex. The families differ from each other by as much as 500 calories per day in the resting metabolic rate. We have also observed that the variability in the 24-hr energy expenditure is greater than that attributable to differences in metabolic body size, age, or sex. It appears, therefore, that there is individual variation in 24-hour energy expenditure. Preliminary analyses indicate that the 24-hour energy expenditure is also a familial characteristic. Furthermore the degree of spontaneous physical activity within the chamber is also a familial characteristic. The tendency for differences in daily caloric expenditure to aggregate in families appears to be largely due to this difference in spontaneous physical activity or what we have termed "fidgeting". These data have also indicated that due to the considerable variation in daily caloric expenditure, recommendations for daily energy requirements cannot be based solely upon body size, age, and sex.

A sufficient number of Caucasian subjects have not yet been examined to determine whether there are differences in caloric expenditure to allow us to determine whether there are differences in caloric expenditure over 24-hour periods between racial groups. However, we have observed some unusual and unexpected findings in that there is a subpopulation of Pima Indians who have an extremely slow metabolic rate during sleep. The reasons for this are unknown, but warrant further study and explanation.

Lipoprotein Metabolism

The Pima Indians have an extremely high prevalence of obesity and NIDDM (as described above), but they have a surprisingly low plasma cholesterol concentrations and reduced low density lipoprotein concentrations and high density lipoprotein concentrations. There is also a low incidence of cardiovascular disease among the nondiabetics. Investigations have been performed to determine how lipoprotein concentrations are regulated in this Indian population such that despite the high prevalence of obesity, lack of activity, and high fat diets, there is the low plasma lipoprotein concentrations and reduced risk of cardiovascular disease.

Using combined VLDL and LDL turnover studies and a multi-compartmented model that was developed for analysis of the kinetic data, VLDL, IDL, and LDL apoB metabolism has been measured in Indians and Caucasians of varying body weights. The results indicate that the low LDL in Pima Indians is related both to a higher fractional catabolic rate for LDL and a higher proportion of VLDL metabolized without conversion to LDL. This suggests that a large flux of substrates and regulators (for example, insulin) causes overproduction of lipoproteins but that compensatory mechanisms are operative that result in maintenance of the low plasma concentrations.

Other studies have indicated that there is a lack of sex difference in HDL concentration in the Pimas. To explain this phenomenon, HDL composition, lipase activities, and steroid hormone concentrations were investigated. The data suggest that obesity has a stronger influence on both the concentrations and composition of HDL in women. The change in HDL in obese women was associated with decreases in levels of HDL subfractions and increased hepatic lipase activity. The net result is that in this obese population of Pima Indian women, the HDL levels are on average low and do not differ those of the men.

The mechanisms associated with the increased VLDL and decreased HDL concentrations associated with non-insulin dependent diabetes mellitushave been investigated. Diabetic Pimas compared to nondiabetics, as in other populations, have an increased risk for cardiovascular disease. Studies on VLDL metabolism and lipase activities were conducted in diabetics before and after sulfonylurea therapy. The data suggested that diabetics have abnormal VLDL and that diabetes affects VLDL triglyceride production independent of that of apoB metabolism, possibly through elevations of free fatty acids or glucose. Improvement of glycemic control was associated with a reversal of abnormalities of VLDL composition and HDL subfractions. Combined studies of VLDL and LDL metabolism in another group of diabetics indicated LDL concentrations in diabetics were influenced by two opposing changes - increased direct removal of VLDL and a decrease in the fractional catabolic rate of LDL. Thus, despite minimal changes in concentration of LDL in diabetics, there are major shifts in metabolic patterns which may explain the increased frequency of atherosclerosis among the diabetics. Further studies are underway to characterize the effects and mechanisms of changes in lipoprotein metabolism in diabetics placed on high carbohydrate, low saturated fat diets.

BIOSTATISTICS AND DATA MANAGEMENT SECTION

Biostatistics and Data Management Section, is a resource for the Diabetes and Arthritis Epidemiology Section and the Clinical Diabetes and Nutrition Section providing computer services, data management, and statistical consulting. Each member of this section collaborates with other investigators in the Branch in providing these services. Their individual scientific accomplishments are reported in the corresponding section reports.

A major activity during the current year has been further work on the Phoenix Clinical Information Section, which is being developed in conjunction with the Data Management Branch of the Division of Computer Research and Technology. While originally it had been expected that this new information system would be operational in the middle of the current year, implementation of the system was delayed as a result of budgetary restraints. While the development work is believed to be essentially complete it is now anticipated that the system will be activated next year.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 69000-21 PECR
Formerly:
Z01 AM 69000-20 PECR

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Diabetes Mellitus and Other Chronic Diseases in the Gila River Indian Community

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W.C. Knowler	Chief	DAES, NIDDK
Others:	P.H. Bennett	Chief	PECRB, NIDDK
	D.J. Pettitt	Assistant Chief	DAES, NIDDK
	J.E. Everhart	Staff Fellow	DAES, NIDDK
	K. Slaine	Staff Fellow	DAES, NIDDK
	R.G. Nelson	Staff Fellow	DAES, NIDDK

COOPERATING UNITS *(if any)*
Biostat. and Data Management Sec., Clinical Diabetes and Nutrition Sec., PECRB, NIDDK; Indian Health Service; Ariz. State U.; State U. of New York at Buffalo; U. of Missouri, Columbia; U. of New Mexico, Albuquerque; Lund University, Sweden

LAB/BRANCH
Phoenix Epidemiology and Clinical Research Branch

SECTION
Diabetes and Arthritis Epidemiology Section

INSTITUTE AND LOCATION
NIDDK, NIH, Phoenix, Arizona 85014

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.2	3.0	2.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Genetic and environmental risk factors for diabetes and vascular complications of diabetes have been studied in the Pima Indians. The residents of the study area, currently numbering approximately 5000 people, have participated in a longitudinal population study for the last 21 years, allowing observations of the natural history of diabetes mellitus and its complications. Nondiabetic subjects with impaired glucose tolerance (IGT) are at high risk of deteriorating to diabetes (27% in 5 years and 48% in 10 years). Decompensation from IGT to diabetes was most likely in those with high fasting insulin concentrations (suggesting insulin resistance) and low two-hour insulin responses to an oral glucose challenge (suggesting impaired insulin responsiveness), consistent with the hypothesis that there are two defects contributing to the development of diabetes: insulin resistance and impaired insulin secretion. Other risk factors for diabetes include obesity, and it was determined from long-term followup of nondiabetic subjects that those with obesity of long duration have a greater incidence of diabetes than the recently obese who have had rapid weight gain.

Risk factors for end stage renal disease were investigated in diabetic Pima Indians. Fasting plasma glucose concentration, duration of insulin use, higher blood pressure, the number of urinary tract infections, prescription of aspirin or indomethicin, and ethanol abuse were predictive factors for this lethal complication of diabetes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 69001-17 PECR
Formerly:
Z01 AM 69001-16 PECR

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Complications and Outcome of Diabetic and Prediabetic Pregnancies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D.J. Pettitt	Assistant Chief	DAES, NIDDK
Others:	P.H. Bennett	Chief	PECRB, NIDDK
	H.R. Baird	Mathematician	BDMS, NIDDK
	W.C. Knowler	Chief	DAES, NIDDK
	K.R. Slaine	Staff Fellow	DAES, NIDDK
	J.E. Everhart	Staff Fellow	DAES, NIDDK

COOPERATING UNITS *(if any)*
Indian Health Service; Biostatistics and Data Management Section, PECRB.

LAB/BRANCH
Phoenix Epidemiology and Clinical Research Branch

SECTION
Diabetes and Arthritis Epidemiology Section

INSTITUTE AND LOCATION
NIDDK, NIH, Phoenix, Arizona 85014

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.6	1.8	0.8

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Diabetes during pregnancy affects the pregnant woman and her offspring as toxemia and cesarean section are both more common in women with diabetes during pregnancy, and malformations, macrosomia, prematurity and perinatal mortality are more common in infants of diabetic mothers. Also, offspring of diabetic women are at an increased risk of developing obesity and glucose intolerance during childhood and young adulthood. The purposes of the project are to identify diabetes and impaired glucose tolerance during pregnancy in women in the Gila River Indian Community, to determine the effects of abnormal glucose tolerance on outcome of the pregnancy, and to determine long term prognosis for the women and their offspring. The diabetes status of every woman is determined at two-yearly intervals and during the third trimester of each pregnancy. The characteristics of women who have diabetes or impaired glucose tolerance during the pregnancy are compared to those of women who are normal during the pregnancy and subsequently develop diabetes and to those of women who remain normal. At birth, cord blood is collected for determination of glycosylated fetal hemoglobin, C-peptide and proinsulin, and maternal blood is also collected for glycosylated hemoglobin. These women and their offspring are followed at two-yearly intervals. It has been previously reported that offspring of diabetic women have more diabetes and more obesity than offspring of nondiabetic and prediabetic women. Birth weight was predictive of relative weight in the 5-9 and 10-14 year old offspring of nondiabetic women but not in the older age group. In contrast, for offspring of prediabetic and diabetic women, birth weight was not predictive of subsequent obesity at any age studied. Offspring of diabetic women were heavier than offspring of nondiabetic and prediabetic women regardless of birth weight. Thus, maternal diabetes was important in predicting body size in the offspring even after accounting for the effects of the birth weight and maternal body size.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 69003-13 PECR
Formerly:
Z01 AM 69003-12 PECR

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Muscle Capillary Basement Membrane Thickness Prior to Onset of Diabetes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P.H. Bennett	Chief	PECRB, NIDDK
Others:	N.B. Rushforth	Chairman	Department of Biology, Case Western Reserve Univ.
	M.D. Siperstein	Vice Chairman	Department of Medicine, Univ. of California at San Francisco
	C. Bogardus	Chief	CDNS, NIDDK
	W.C. Knowler	Chief	DAES, NIDDK

COOPERATING UNITS *(if any)*

Department of Biology, Case Western Reserve University, Cleveland, Ohio
Department of Medicine, University of California, San Francisco, California

LAB/BRANCH
Phoenix Epidemiology and Clinical Research Branch

SECTION
Diabetes and Arthritis Epidemiology Section

INSTITUTE AND LOCATION
NIDDK, NIH, Phoenix, Arizona 85014

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.3	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Several metabolic and morphologic changes have been claimed to precede the onset of diabetes, including changes in the pattern and quantity of insulin secretion and alteration in the thickness of capillary basement membranes. Some 10 years ago, Pima Indians with two diabetic parents, and with neither parent diabetic received oral and intravenous glucose tolerance tests, and a biopsy of the quadriceps muscle from which quantitative determinations of the thickness of the capillary basement membrane were made. The same subjects are being reexamined to determine if their insulin secretion or the thickness of their capillary basement membrane predicted the subsequent development of diabetes, and to determine if there was differential thickening of the muscle capillary basement membrane with increasing age in those with diabetic parents compared to those without. The results will help to determine if vascular lesions at the level of the capillary are present before hyperglycemia develops.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 69006-16 PECR
Formerly:
Z01 AM 69006-15 PECR

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Gila River Indian Community Autopsy Study

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P.H. Bennett	Chief	PECRB, NIDDK
Others:	W.C. Knowler	Chief	DAES, NIDDK
	D.J. Pettitt	Assistant Chief	DAES, NIDDK
	M.L. Sievers	Guest Researcher	DAES, NIDDK
	D. Althaus	Pathologist	Phoenix Indian Medical Center
	L. Orci	Chairman	Dept. of Morphology, University of Geneva

COOPERATING UNITS *(if any)*

Pathology Department, Phoenix Indian Medical Center, Phoenix, Arizona; Dept. of Morphology, University of Geneva, Geneva, Switzerland

LAB/BRANCH
Phoenix Epidemiology and Clinical Research Branch

SECTION
Diabetes and Arthritis Epidemiology Section

INSTITUTE AND LOCATION
NIDDK, NIH, Phoenix, Arizona 85014

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.3	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The postmortem characteristics of Pima Indians of the Gila River Indian Community are investigated so that findings in subjects with and without diabetes mellitus can be correlated with studies in living subjects. Medical records are reviewed for the determination of cause of death and for the occurrence of certain serious diseases or complications of diabetes.

PHS 6040 (Rev. 1/84) GPO 914-916

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 69009-21 PECR
Formerly:
Z01 AM 69009-20 PECR

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Natural History of Arthritis and Rheumatism in the Gila River Indian Community

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P.H. Bennett	Chief	PECRB, NIDDK
Others:	D.J. Pettitt	Assistant Chief	DAES, NIDDK
	W.C. Knowler	Chief	DAES, NIDDK
	K.R. Slaine	Staff Fellow	DAES, NIDDK
	J.E. Everhart	Staff Fellow	DAES, NIDDK
	H.R. Baird	Mathematician	BDMS, NIDDK
	A. Del Puente	Visiting Fellow	DAES, NIDDK

COOPERATING UNITS *(if any)*

Biostatistics and Data Management Section, PECRB

LAB/BRANCH
Phoenix Epidemiology and Clinical Research Branch

SECTION
Diabetes and Arthritis Epidemiology Section

INSTITUTE AND LOCATION
NIDDK, NIH, Phoenix, Arizona 85014

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.9	1.3	1.6

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects
  - ☒ (a1) Minors
  - ☐ (a2) Interviews
- ☐ (b) Human tissues
- ☐ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The development and progression of osteoarthrosis, rheumatoid arthritis and ankylosing spondylitis are being determined by means of clinical, radiographic and serological examinations carried out prospectively at two-yearly intervals among adults of the Gila River Indian Community (Pima Indians) in Arizona, in conjunction with epidemiological studies of diabetes in the same community. The purpose of this investigation is to ascertain the determinants of these diseases in the population, and to identify factors which alter the natural history of progression of the disease. Host factors such as age, sex, and various gene markers including HLA and Gm, together with various potential environmental determinants, such as obesity and evidence of exposure to infectious agents, will be studied prospectively to determine their relationship to the development of these diseases. Longitudinal data have now been collected over a 21 year period in the population and represent a unique data set for epidemiological studies of arthritis.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 69013-05 PECR
Formerly:
Z01 AM 69013-04 PECR

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Diabetes, Arthritis and Other Metabolic Diseases in the Pacific Region

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: P.H. Bennett Chief PECRB, NIDDK

COOPERATING UNITS *(if any)*
WHO Collaborating Centre for the Epidemiology of Diabetes Mellitus (P. Zimmet and H. King)
South Pacific Commission (R. Taylor)

LAB/BRANCH
Phoenix Epidemiology and Clinical Research Branch

SECTION
Diabetes and Arthritis Epidemiology Section

INSTITUTE AND LOCATION
NIDDK, NIH, Phoenix, Arizona 85014

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The prevalence of diabetes and its associated vascular complications have been assessed in several Pacific Island populations, including Polynesians and Melanesians, living in traditional ways as well as in urbanized communities.

In general, much higher prevalences of diabetes and the associated complications were found in the urbanized populations, and attempts to determine the reasons for these differences are being pursued. Increased obesity, reduced physical activity, changes in dietary composition and intake appear to contribute to these differences in frequency, but genetic factors also are likely important in determining the frequency of the diabetes, itself, and possibly the type and frequency of associated complications. Identification of the relative importance of environmental determinants of diabetes is a prerequisite to formulating preventive measures for this disease in developing countries.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 69014-09 PECR
Formerly:
Z01 AM 69014-08 PECR

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Lipoprotein Composition and Metabolism in Pima Indians

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B.V. Howard	Associate Chief	CDNS,NIDDK
Others:	W. Abbott	Visiting Scientist	CDNS,NIDDK
	P.H. Bennett	Chief	PECRB,NIDDK
	W.C. Knowler	Chief	DAES,NIDDK

COOPERATING UNITS *(if any)*
Indian Health Service
S.M. Grundy,Dept. of Medicine,Univ. of Texas,S.W. Medical School,Dallas,TX
W. Beltz,Dept. of Medicine,Univ. CA San Diego,Medical School,La Jolla,CA

LAB/BRANCH
Phoenix Epidemiology and Clinical Research Branch

SECTION
Clinical Diabetes and Nutrition Section

INSTITUTE AND LOCATION
NIDDK, NIH, Phoenix, Arizona 85016

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.6	0.2

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Pima Indians have a high prevalence of obesity, diabetes mellitus, and hyperinsulinemia, but they have low plasma cholesterol levels, reduced low density lipoprotein (LDL) and high density lipoprotein (HDL), and decreased incidence of cardiovascular disease (CVD). Lipoprotein composition and metabolism in Pima Indians are being investigated in order to further understand control of lipoprotein metabolism and how lipoproteins are related to obesity and CVD. A multicompartmental model has been developed for the analysis of kinetic data for the simultaneous measure of VLDL, IDL, and LDL apoB metabolism. Comparison of apoB metabolism in Pimas and weight-matched Caucasians indicated that the low LDL in Pimas is related to both higher FCR for LDL and higher proportion of VLDL metabolized without conversion to LDL. The results of lipoprotein metabolism studies suggest a large flux of substrates and regulators (e.g., insulin) which cause overproduction of lipoproteins, but that compensatory mechanisms are operative which result in maintenance of low plasma concentrations.

In order to understand the reason for the lack of sex differences in HDL in the Pimas, we have studied HDL composition, lipase activities, and steroid hormone concentrations. The data indicated that obesity in this population has a stronger influence on both concentrations and composition of HDL in women. The change in HDL in obese women was associated with decreases in levels of plasma estradiol and increases in hepatic lipase activities. Since there are so few lean women in the Pima population, the net result is that HDL levels in women in the population as a whole do not differ from those of men.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
ZO1 DK 69015-04 PECR
Formerly:
ZO1 AM 69015-03 PECR

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cross-sectional and longitudinal studies of "pre-diabetes" in the Pima Indians

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. Bogardus	Chief	CDNS,NIDDK
Others:	B.V. Howard	Associate Chief	CDNS,NIDDK
	J.E. Foley	Senior Scientist	CDNS,NIDDK
	D.M. Mott	Research Chemist	CDNS,NIDDK
	S. Lillioja	Visiting Associate	CDNS,NIDDK
	J. Zawadzki	Medical Officer	CDNS,NIDDK
	A. Young	Visiting Associate	CDNS,NIDDK
	W. Abbott	Visiting Scientist	CDNS,NIDDK

COOPERATING UNITS *(if any)*

Indian Health Service

LAB/BRANCH
Phoenix Epidemiology and Clinical Research Branch

SECTION
Clinical Diabetes and Nutrition Section

INSTITUTE AND LOCATION
NIDDK, NIH, Phoenix, Arizona 85016

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.5	4.6	2.9

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Pima Indians have the highest reported prevalence and incident rate of non-insulin dependent diabetes mellitus (NIDDM) of any population of the world. The diabetes occurs more frequently in the offspring of diabetic mothers than in the offspring of non-diabetic parents. The reasons for this are unknown. In this project, we are longitudinally studying Pima Indians to determine the sequence of metabolic events that occurs with the development of NIDDM and also to isolate the predictor of the development of NIDDM. Studies are done on a yearly basis on the adult offspring of diabetic mothers and of non-diabetic parents to characterize their insulin and carbohydrate metabolism both in vivo and in vitro. Data collected to date has clearly demonstrated for the first time the relationships between degree of obesity as determined by careful body composition studies with insulin resistance. Obesity accounts at best for only about 50% of the variance in insulin resistance. Physical fitness, as determined by maximal oxygen uptake, appears to account for about 25% of the variance in insulin resistance. Most of insulin resistance appears to be due to a reduction in insulin-mediated glucose storage rather than a reduction in insulin-mediated glucose oxidation. Preliminary longitudinal analyses of the data so far have indicated that the development of impaired glucose tolerance is associated with weight gain, and decreased insulin action for glucose storage, but with little change in insulin action in isolated adipocytes from the same subjects. Approximately 14 subjects have developed diabetes and an equal number have developed impaired glucose tolerance. Statistical analyses are now underway to isolate the metabolic characteristic that is most predictive of these deteriorations of glucose tolerance.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 69016-03 PECR
Formerly:
Z01 AM 69016-02 PECR

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Control of glucose transport in insulin sensitive tissues

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.E. Foley	Senior Scientist	CDNS,NIDDK
Others:	K. Kubo	Visiting Associate	CDNS,NIDDK

COOPERATING UNITS *(if any)*

Indian Health Service

LAB/BRANCH
Phoenix Epidemiology and Clinical Research Branch

SECTION
Clinical Diabetes and Nutrition Section

INSTITUTE AND LOCATION
NIDDK, NIH, Phoenix, Arizona 85016

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have previously shown that glucose transport is the rate limiting step in glucose uptake and metabolism up to 2 mM glucose in the presence of a maximum insulin concentration and up to 7 mM glucose in the absence of insulin. We now have found that in the presence of insulin above 2 mM glucose, glycogen synthesis, and lactate synthesis become the rate limiting steps. In the absence of insulin above 7 mM glucose, the rate limiting step became lactate synthesis.

We now have found that rats made diabetic by streptozotocin have a reduced capacity for glucose transport in the presence and absence of insulin. The glucose concentration over which the rate limiting step changes from glucose transport to a step beyond glucose transport does not change.

We are also studying the effect of a high fat diet which has previously been shown to decrease glucose transport and metabolism in adipocytes to see whether muscle transport is similarly decreased and whether this influences the glucose concentration at which transport is no longer rate limiting.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 69017-03 PECR
Formerly:
Z01 AM 69017-02 PECR

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Metabolic effects of weight loss in non-insulin dependent diabetes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. Bogardus	Chief	CDNS,NIDDK
Others:	J. Zawadzki	Medical Officer	CDNS,NIDDK
	J.E. Foley	Senior Scientist	CDNS,NIDDK

COOPERATING UNITS *(if any)*
Indian Health Service
Burns Institute,Galveston,TX (R. Wolfe)

LAB/BRANCH
Phoenix Epidemiology and Clinical Research Branch

SECTION
Clinical Diabetes and Nutrition Section

INSTITUTE AND LOCATION
NIDDK, NIH, Phoenix, Arizona 85016

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Non-insulin dependent diabetes mellitus (NIDDM) is characterized by abnormalities of insulin secretion and insulin action. Previous studies have indicated some improvement in insulin action in vivo with weight loss in these subjects. It has not been clear, however, whether the changes in insulin action are due to changes in the sensitivity of the insulin sensitive processes or to a change in the capacity for glucose metabolism. To assess the mechanisms of the improvement of insulin action in subjects with NIDDM, we have performed a series of measures of in vivo insulin action to assess insulin sensitivity and capacity for glucose metabolism in subjects with NIDDM before and after weight loss. We also performed in vitro studies of isolated abdominal adipocytes from the same subjects. In addition, it has been known for some time that the reduction in glycemia in subjects with NIDDM after weight loss is associated with a reduction of the rate of hepatic glucose production. It is possible that the decrease in hepatic glucose production in these subjects is, in part, due to a reduction in the rate of the glucose recycling through the Cori cycle. Our results show that the improvement in insulin action in subjects with NIDDM after weight loss is the result of an improvement in the capacity for insulin action rather than a change in insulin sensitivity in vivo. There were no associated changes in isolated adipocytes to accompany the changes that were observed in in vivo insulin action. Also, we observed increased rates of the Cori cycle, as measured by 1-C-^{13}-glucose recycling, in subjects with NIDDM which tended to decrease with weight loss but did not return to the levels observed in equally obese subjects with normal glucose tolerance.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 69018-03 PECR
Formerly:
Z01 AM 69018-02 PECR

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Lipoprotein metabolism in diabetes and the effects of therapy

PRINCIPAL INVESTIGATOR *(List other professionel personnal below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B.V. Howard	Associate Chief	CDNS,NIDDK
Others:	W. Abbott	Visiting Scientist	CDNS,NIDDK

COOPERATING UNITS *(if any)*
Indian Health Service
2nd Dept. of Medicine,Univ. of Helsinki School of Medicine,Helsinki,Finland
Dept. of Medical Education,Good Samaritan Medical Center,Phoenix,Arizona
Dept of Medicine,Institute San Raffaele,Univ. of Milan,Italy

LAB/BRANCH
Phoenix Epidemiology and Clinical Research Branch

SECTION
Clinical Diabetes and Nutrition Section

INSTITUTE AND LOCATION
NIDDK, NIH, Phoenix, Arizona 85016

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.7	0.6	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The increased VLDL and decreased HDL commonly associated with non-insulin dependent diabetes are of concern because of their possible role in the etiology of the greatly increased cardiovascular disease in this disorder. Moreover, it has not been established how therapy of diabetes, with either diet or oral hypoglycemic agents, influences lipoprotein metabolism. This study compares VLDL and LDL metabolism, fatty acid metabolism, and lipoprotein lipase activities in type II diabetics and in age and weight-matched non-diabetics. Studies were also conducted in diabetics before and after therapy with sulfonylureas and also diabetics are being compared on high and low fat diets. The data suggest that diabetics have abnormal VLDL and that diabetes influences VLDL-TG production independent of that of apoB, possibly through elevations of free-fatty acids or glucose. LDL concentrations in diabetics are influenced by two opposing changes - increase in direct removal of VLDL, but decrease in FCR for VLDL. Improvement of glycemic control is followed by significant falls in VLDL-TG and LDL cholesterol and reversal of abnormalities of HDL subfractions and VLDL composition. Transfer to a high carbohydrate, low saturated fat diet leads to decreases in LDL and no change in HDL. This was accompanied by no change in VLDL in most diabetics; two brothers, however, showed significant increases in VLDL and glucose. indicating that diabetics must be carefully monitored when transferred to high carbohydrate regimens.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 69019-03 PECR
Formerly:
Z01 AM 69019-02 PECR

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Free-fatty acid metabolism and obesity: in vivo, in vitro comparisons

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B.V. Howard	Associate Chief	CDNS,NIDDK
Others:	S. Lillioja	Visiting Associate	CDNS,NIDDK
	C. Bogardus	Chief	CDNS,NIDDK
	J.E. Foley	Senior Scientist	CDNS,NIDDK

COOPERATING UNITS *(if any)*
Indian Health Service

LAB/BRANCH
Phoenix Epidemiology and Clinical Research Branch

SECTION
Clinical Diabetes and Nutrition Section

INSTITUTE AND LOCATION
NIDDK, NIH, Phoenix, Arizona 85016

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Free-fatty acid release from adipose tissue is the sole mechanism for the mobilization of fat cell triglyceride. Defective FFA release in obese subjects will result in the maintenance of large fat stores and the perpetuation of obesity. Excessive FFA release on the other hand might lead to increased lipoprotein formation or a worsening of reduced glucose disposal. We have used FFA turnover and lipid oxidation rate, assessment of body composition to measure total fat mass, and in vitro measurements of lipolytic rates in isolated fat cells, in the same subjects to investigate possible mechanisms of regulation of in vivo FFA metabolism. The data indicate that fatty acid availability or use per kg of fat actually decreases with increasing obesity. This could be due to a contribution of nonadipose tissue triglyceride to the FFA turnover and lipid oxidation. Even more likely, however, is that plasma or local tissue factors in obese individuals prevent fatty acid from becoming available in spite of increased stores. This was also suggested by the findings in vitro. These data suggested that the fat cells were more than capable of releasing large amounts of FFA in obese subjects but apparently failed to do so in vivo. These results indicate that in obese subjects much of the increased fat store may not be accessible to the rest of the body. Furthermore, in vitro measurements of fat cell lipolysis cannot be used to directly predict in vivo fatty acid metabolism. Finally, these data indicate that there is a large non-oxidative fatty acid disposal that may be important in the regulation of the plasma FFA concentration.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 69020-03 PECR
Formerly:
Z01 AM 69020-02 PECR

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Muscle glycogen synthase activity and insulin-mediated glucose disposal

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. Bogardus	Chief	CDNS,NIDDK
Others:	D. Mott	Research Chemist	CDNS,NIDDK
	A. Young	Visiting Associate	CDNS,NIDDK
	S. Lillioja	Visiting Associate	CDNS,NIDDK
	H. Yki-Jarvinen	Visiting Fellow	CDNS,NIDDK

COOPERATING UNITS *(if any)*
Indian Health Service

LAB/BRANCH
Phoenix Epidemiology and Clinical Research Branch

SECTION
Clinical Diabetes and Nutrition Section

INSTITUTE AND LOCATION
NIDDK, NIH, Phoenix, Arizona 85016

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.9	0.7	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Most of an oral glucose load is taken up by muscle in man. We have previously demonstrated the importance of carbohydrate storage in distinguishing between individuals with low and high insulin-mediated glucose disposal. Reduced insulin-mediated glucose storage is associated with a reduced muscle glycogen synthase activity in man. In our effort to further clarify the importance of the regulation of muscle glycogen synthase to the regulation of insulin-mediated glucose storage, we have observed the following: insulin activation of glucose storage and glycogen synthase have similar ED_{50} values. Subjects with low insulin-mediated glucose disposal rates have dose-response curves for both glycogen synthase and glucose storage which are shifted to the right (lower sensitivity) and have reduced capacity. Diabetic subjects have reduced fasting muscle glycogen concentrations and reduced total glycogen synthase activity. Non-diabetic subjects show a negative correlation between fasting plasma glucose and their muscle glycogen content and their total glycogen synthase activity (active plus inactive forms). These results suggest that glycogen synthase activity is closely associated with the process of glucose disposal and that alterations in the regulation of the enzyme coincide with the altered glucose storage observed in subjects with low insulin-mediated glucose storage rates. The elevated G-6-P content in muscle from subjects with low insulin-mediated glucose disposal rates indicates that the most significant reduction in their glucose metabolism occurs post-G-6-P. In addition, preliminary results suggest that a large fraction of stored glucose ends up as muscle glycogen. Taken together, these results suggest that abnormal insulin regulation of glycogen synthase may account for a large part of the reduced insulin-mediated glucose disposal.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 69021-06 PECR
Formerly:
Z01 AM 69021-05 PECR

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Thermogenesis in Pima Indians: possible cause for obesity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: C. Bogardus	Chief	CDNS,NIDDK
Others: E. Ravussin	Visiting Scientist	CDNS,NIDDK

COOPERATING UNITS *(if any)*
Indian Health Service

LAB/BRANCH
Phoenix Epidemiology and Clinical Research Branch

SECTION
Clinical Diabetes and Nutrition Section

INSTITUTE AND LOCATION
NIDDK, NIH, Phoenix, Arizona 85016

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	1.2	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Pima Indian population of Arizona has one of the highest reported prevalence rates of obesity in the world. To determine whether a "thrifty gene" is the genetic defect predisposing the Pima Indians to obesity, we have investigated different components of the total daily energy expenditure in both Pima Indians and Caucasians. Basal metabolic rates, glucose-induced thermogenesis, and response to exercise have been measured by an open-circuit hood system indirect calorimeter, whereas our new respiratory chamber has been used to measure the major determinants of 24-hour energy expenditure. Furthermore, we are planning to use this chamber to investigate the short-term response to over- and underfeeding in terms of energy expenditure and substrate oxidation in lean and obese Pima Indians. Measurements of different components of energy expenditure have been performed in siblings in order to assess the importance of genetic factors in the overall energy balance.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 69022-05 PECR
Formerly:
Z01 AM 69022-04 PECR

PERIOD COVERED
October 1, 1985 through March 31, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Pathophysiology of the adipocyte in human obesity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.E. Foley	Senior Scientist	CDNS,NIDDK
Others:	B.V. Howard	Associate Chief	CDNS,NIDDK
	W.G. Abbott	Visiting Scientist	CDNS, NIDDK

COOPERATING UNITS *(if any)*
Indian Health Service
Laboratory of Cellular Metabolism and Obesity,NIDDK (S.W. Cushman)
Sandoz Laboratories, East Hanover,NJ (L.B. Salans)

LAB/BRANCH
Phoenix Epidemiology and Clinical Research Branch

SECTION
Clinical Diabetes and Nutrition Section

INSTITUTE AND LOCATION
NIDDK, NIH, Phoenix, Arizona 85016

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 69023-01 PECR

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*

Skeletal muscle morphology as a determinant of in vivo "insulin resistance" in man

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. Bogardus	Chief	CDNS,NIDDK
Others:	S. Lillioja	Visiting Associate	CDNS,NIDDK

COOPERATING UNITS *(if any)*

Indian Health Service
Dept. of Physical Education,University of Texas,Austin,TX (J.L. Ivy)

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Clinical Diabetes and Nutrition Section

INSTITUTE AND LOCATION

NIDDK, NIH, Phoenix, Arizona 85016

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.3	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In vivo resistance to the action of insulin on glucose disposal is commonly found in obese subjects. The mechanism for this does not appear to be solely an inhibition of glucose metabolism by fatty acids released from the enlarged fat stores. This has lead us to assess the role of obesity associated changes in skeletal muscle in in vivo "insulin resistance", since glucose disposal after an oral or IV glucose load is into this tissue. We have assessed the relationship of insulin resistance, degree of obesity, and skeletal muscle morphology in Pima and Caucasian non-diabetic men. We have found a significant correlation between capillary density in skeletal muscle and in vivo insulin action. We suggest that the increased diffusion distances created by muscle cell enlargement are part of the mechanism by which obesity is associated with "insulin resistance". Furthermore, the finding may explain the nonlinear relationship of insulin action and degree of obesity. The association of increased plasma glucose with obesity and capillary density may reflect a compensatory mechanism to overcome reduced glucose disposal. This compensatory mechanism has the potential, however, for producing adverse effects if prolonged. We conclude the changes in fat-free mass induced by obesity are as important metabolically as changes that occur in the fat compartment.